Production of Microalgal Concentrates for Aquaculture (Part 1: Algae Culture)

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OBJECTIVES[1]:

1. to evaluate the suitability of the BIOCOIL-type tubular photobioreactor for the culture of algal species identified as being of importance to hatcheries.

2. to determine the optimal large-scale (10,000 to 1,000,000 L) culture system for each algal species and evaluate the economic feasibility.

3. to determine the best harvesting and concentration method for each algal species.

4. to determine the best manner of further processing, preservation, packaging, storage and transport of concentrates of each algal species and blends thereof.

5. to carry out growth and survival studies in which pilot production packs of selected single species algal concentrates or blends thereof are compared with equivalent algae culture diets (as controls) under commercial hatchery and nursery conditions

6. to prepare a business plan for commercialisation

7. to prepare user-guidelines to facilitate adoption and efficient utilisation of algal concentrates

The ultimate objective was to assist the Australian aquaculture industry by providing a reliable source of a high quality, reasonably priced microalgal concentrates. Furthermore, such products also have international markets, thus providing a new export opportunity for Australian aquaculture.

The targets for this project were:

- nutritionally superior algal concentrates with a shelf-life of 12 - 30 weeks.
- a cost for the algal concentrate of less than \$A 30 kg^{-1}.

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[1]*Objectives 3, 4, 5 and 7 will be reported on in a separate report prepared by Dr Mike Heasman (NSW Fisheries) as the algal concentrate part of the project was extended as another FRDC funded project (No: 96/342) and it is more logical to provide a single complete report on this work. The present report only covers objectives 1, 2 and 6.*

NON TECHNICAL SUMMARY:

Microalgae are an important food source and feed additive in the commercial rearing of many aquatic animals, especially the larvae and spat of bivalve molluscs, penaeid prawn larvae and live food organisms such as rotifers. The importance of algae in aquaculture is not surprising as algae are the natural food source of these animals. Although several alternatives for algae exist such as yeasts and microencapsulated feeds, live algae are still the best and the preferred food source.

The main constraint on microalgal production for aquaculture is the cost. For example, a survey of Australian hatcheries conducted by us shows that they estimate that, on average, 30 - 40% (max. 70%) of hatchery costs can be attributed to algal culture. Estimates of the cost of aquaculture based on surveys of Australian ond overseas hatcheries range from about $80 to $800 per kilogram dry weight.

This cost is extremely high when compared with the costs of other commercial large-scale algal producers. The production costs of algae such as *Spirulina, Dunaliella* and *Chlorella* are of the order of $\$10\text{-}\$50.kg^{-1}$ dry algae. The reasons for this high cost of algae for aquaculture relate to the species of algae cultured, the rather inefficient culture systems and to a large extent, the small scale of the algal culture systems used in hatcheries. The lowest algae production costs are achieved only in very large hatcheries.

In order to reduce the costs of algae to hatcheries there is therefore the need to develop more efficient and reliable culture systems and to achieve economics of scale by developing larger centralised algae culture facilities which can then deliver the algae to the hatcheries as required. This involves the ability to concentrate the algae and to be able to store and ship them without compromising the nutritional qualities of the algae. This project addresses both of these problems by adapting a new type of algal culture system, the BIOCOIL which is a helical tubular photobioreactor, and by developing improved methods of concentrating and storing the algae.

Reliable long-term culture in a helical tubular photobioreactor of several species of marine microalgae used in the aquaculture industry was achieved. The basic design of the helical tubular photobioreactor, the BIOCOIL, was adapted and improved in order to be able to grow these fragile algae which could not be grown in the standard BIOCOIL design. Several small (34 - 80L) laboratory BIOCOILs were constructed or adapted for these experiments. An important feature of the design were airlifts as the means of circulating the algal culture. Based on experience with these BIOCOILs two pilot-scale (approx. 550L) BIOCOILs were designed and constructed.

The microalgae which grow best in the BIOCOILs were *Isochrysis galbana* (T-iso), *Tetraselmis suecica, T. chuii, Pavlova lutheri* and *Chroomonas salina.* The diatom *Skeletonema costatum* was also grown successfully in the laboratory, but cell yields were low and thus this alga was not tested in the large BIOCOILs. It was found that *Skeletonema* required regular addition of Si in order to avoid culture 'crashes'. The other two diatoms, *Chaetoceros gracilis* and *C. calcitrans* were not grown successfully.

The algae were initially grown indoors in the small BIOCOILs in order to develop appropriate protocols for successful long-term culture (long-term equals at least 2 months of reliable culture). The algae were grown in semi-continuous mode with periodical harvesting of part of the culture followed by the addition of fresh medium. After successful laboratory culture the algae were

grown outdoors in natural daylight in a glasshouse in the small BIOCOILs before being scaled-up to the 550L pilot-scale BIOCOILs. In almost all cases the cultures were stable for at least two months and generally longer than that. The maximum length that a culture can be maintained remains to be determined as the cultures were generally terminated after 2 to 3 months so that other species could be examined. The longest culture period was about 155 days.

Some of the species also showed some sticking to the inside of the tubes of the photobioreactor. However, the sticking could be reduced by adding fresh medium more often. In normal operational mode for the production of algae for aquaculture the cultures would be harvested regularly and the medium therefore would be replaced more often than was the case in some of these experiments. This would also reduce sticking of the algae.

The maximum cell density achieved outdoors in the BIOCOILs for *Tetraselmis, Isochrysis, Pavlova* and *Chroomonas* was usually greater than 1 g dry wt.L^{-1} and the estimated productivity in a production size BIOCOIL (2,000 to 10,000L) is better than 0.5 g dry wt.L^{-1}. day^{-1} under Perth climatic conditions. At these productivities the BIOCOIL is commercially viable for large hatcheries.

KEYWORDS: photobioreactor, *Tetraselmis, Chaetoceros, Skeletonema, Pavlova, Isochrysis, Chroomonas*, algae culture.

BACKGROUND AND NEED

Microalgae are essential for the commercial rearing of many aquatic animals, especially the larvae and spat of bivalve molluscs, penaeid prawns and live food organisms such as rotifers which are used in turn to rear larval marine finfish and crustaceans.

In recent years extensive studies have been undertaken to determine the nutritional requirements of the target species and the chemical composition of the possible algae which can be used as a food source (e.g. Volkman *et al.*, 1981, 1989, 1991, 1993; Brown, *et al.*, 1989, 1993; Brown, 1991; Brown, & Jeffrey, 1992; Brown, & Miller, 1992; Dunstan *et al.*, 1992, 1994; DeRoeck-Holtzhauer *et al.*, 1993; Brown, & Farmer, 1994) and these studies provide an excellent data-base for the selection of algal species for use in aquaculture. However, and understanding of the biochemical composition is not enough, data are also required on the ability of the target animal to feed on the algae, on the digestibility of the algae and on the nutritional requirements of the animal species being cultured. As well as this we need data on whether the algal species can be cultured reliably and cost-effectively on a large scale. microalgae culture still presents major problems and challenges for the aquaculture industry:

- The main constraint on microalgal production for aquaculture is the cost. For example, a survey of Australian hatcheries conducted by us shows that they estimate that, on average, 30 - 40% (max. 70%) of hatchery costs can be attributed to algal culture. The best US estimate gives a cost of greater than \$US 50.kg^{-1} of dry algal biomass (Fulks & Main, 1991) for a large, specialised oyster hatchery. However, for many hatcheries, especially smaller hatcheries, this cost is likely to be much higher. For example, Coutteau and Sorgeloos (1992, 1993) in their world-wide survey of bivalve hatcheries and Epifanio (1979a) give costs of up to \$US 300 - 400.kg^{-1} dry weight of microalgae, and a more recent international survey conducted by us gave costs of up to \$US 600.kg^{-1} for algae production, with the highest costs in small hatcheries.

This cost is extremely high when compared with the costs of other commercial large-scale algal producers. The production costs of algae such as *Spirulina, Dunaliella* and *Chlorella* are of the order of \$US 15-20.kg^{-1} dry algae (Borowitzka, 1991; Tanticharoen *et al.*, 1993). Why then is the cost of microalgae production for aquaculture so much higher? The answer to this question lies in several factors:

- The algal species cultured for aquaculture do not grow in highly selective environments as do *Dunaliella, Spirulina* and *Chlorella,* and are therefore generally cultured in closed systems rather than in open ponds or raceways.

- The climate where the algae are grown is often not optimal for algal growth (too cold, too hot, too much rain etc.) and the algae have to be grown indoors with artificial lighting and temperature control thus increasing energy costs. Artificial lighting also results in lower yields as the cultures are generally light limited.

- Algal culture requires expertise often not found in the hatchery/farm and is often regarded as a diversion of resources resulting in major problems when cultures 'crash'. This reduced reliability increases costs.

- The algae are grown mainly in batch culture which increases the labour required and the capital costs of the facilities.

- The culture systems used (carboys, large bags, tower reactors, tanks etc. (Fox, 1983; De Pauw & Persoone, 1988; Fulks & Main, 1991; O'Meley & Daintith, 1993)) are inefficient, leading to low productivities and less reliable cultures. These systems are also not suitable for computer control or automation, thus increasing labour costs.

- The cost of producing algal biomass is strongly affected by economies of scale (Borowitzka, 1992; Coutteau & Sorgeloos, 1992). The algal requirements of most hatcheries are very small when compared to the scale of commercial *Dunaliella, Spirulina* and *Chlorella* culture operations, thus significantly increasing the unit cost of the algae.

An international workshop on microalgal culture for aquaculture (Fulks & Main, 1991) ranked the primary costs associated with microalgal production in the order of: labour, supplies and chemicals, facilities and energy. The potential solutions to the cost of labour were seen as:

(1) computer control/automation,
(2) streamlining of scale-up,
(3) reduction of the number of culture units,
(4) cryopreservation of stock cultures,
(5) continuous culture, and
(6) use of pre-mixed nutrients.

These solutions however are only a part of the equation and they ignore the wide experience of commercial algae growers. In particular they ignore the potential benefits of using better culture systems and of the use of larger algae culture facilities. This is partially due to the fact that most aquaculturalists perceive algal culture as an in-house activity and have little experience in large-scale algal culture.

The solution to the high costs of algal culture and the associated problems with culture stability, lies in having dedicated algal production units which can use efficient culture techniques, have appropriately trained staff, a Total Quality Management (TQM) programme, and which can achieve economies of scale, thus providing hatcheries with a cheaper, reliable and high quality source of algae.

New culture systems

It is necessary to improve the large-scale algal systems used. Present culture methods used in hatcheries for the production of the required microalgae rely mainly on bag or tank culture (up to 500L per bag) and pond culture (up to 10,000 L) (cf. Costa-Pierce, 1982; Lee & Tamaru, 1993; Liao *et al.*, 1993b). Algal productivity in these culture systems is low and they are known to be unreliable and require very careful management (Sato, 1991). The bag and pond culture systems used represent very primitive technology. In the last decade, major advances have been made in the large-scale culture of microalgae, not only in open systems, but also in closed bioreactors (Tredici & Materassi, 1992; Chaumont, 1993; Borowitzka, 1996) and the aquaculture industry has yet to adopt these new technologies.

The aquaculture industry also uses a range of algal species due to the different nutritional and algae size requirements of the animal species studied. The major species and the animals they are fed to are listed in Table 1.

Table 1. ***Microalgal species commonly used in aquaculture and the animals they are usually fed to.***

Species	Molluscs	Crustaceans	Rotifers
Isochrysis galbana (T-iso)	●		
Chaetoceros muelleri	●		
Chaetoceros calcitrans	●	●	
Skeletonema costatum	●	●	
Thalassiosira pseudonana	●	●	
Tetraselmis spp		●	
Nannochloropsis spp.			●
Pavlova lutheri	●		
Nitzschia & Navicula spp	●[1]		

[1]Used in the culture of abalone

In order to be able to produce 'clean' unialgal cultures closed culture systems are essential. Of the various types of closed bioreactor systems available, the tubular photobioreactor seems to be the most reliable, efficient and cost effective. Simple types of tubular reactors were already proposed for aquaculture some time ago (e.g. Canzonier & Brunetti, 1976) and around the world several research groups are working on different designs (e.g. Chaumont *et al.*, 1988; Tredici & Materassi, 1992; Pulz, 1994; Borowitzka, 1996; Hu *et al.*, 1996). The helical tubular photobioreactor designed and patented by Biotechna Ltd (the BIOCOIL™ - Robinson *et al.*, 1988) so far has proven to be the most effective and long-term laboratory and pilot-scale studies in large outdoor BIOCOILs with several microalgae (*Tetraselmis, Isochrysis, Chaetoceros, Pavlova, Nannochloropsis, Phaeodactylum, Spirulina, Chlorella* etc.) have been carried out in the UK and Australia (Chrismadha & Borowitzka, 1994; Borowitzka, 1996; Watanabe & Hall, 1996). This system not only provides a controlled, contamination-free environment, but it can be used for continuous culture at much higher cell densities than can be achieved with traditional systems. The higher cell densities are mainly a result of being able to work outdoors using natural sunlight. The ability to operate a reliable continuous culture system not only reduced production costs but also provides an algal biomass of consistent quality. The BIOCOIL can also be semi-automated with computer control thus reducing the amount of labour required.

The solution to the high costs of algal culture and the associated problems with culture stability, lies in having dedicated algal production units which can use efficient culture techniques, have appropriately trained staff, a Total Quality Management programme, and which can achieve economies of scale, thus providing hatcheries with a cheaper, reliable and high quality source of algae.

The major barrier to the implementation of such a facility is the need to preserve the algal biomass so that it can be shipped to the hatcheries for use. Dried algae have been available in the past (produced by Celsys Plc), however these heterotrophically grown algae were nutritionally inadequate and their use has caused problems with fouling of the water (Laing & Verdugo, 1991; Numaguchi & Nell, 1991 and a Market Survey conducted by Western Biotechnology). The price of this product was also very high, and it is no longer available. Wet algal concentrates (pastes) appear to be a much better option and some of these are currently available in the US and Canada, but again at a very high cost. Recalculating the sale price of these on a dry weight basis the cost of these concentrates is > \$US 500.kg^{-1}.

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	RANK	
	Range	*Mean*
Nutritional composition	5 - 10	(8.79)
Consistency of supply	5 - 10	(8.79)
Management/production advantages offered	2 - 10	(8.31)
Nutritional consistency	1 - 10	(8.00)
Product to be alive	1 - 10	(7.79)
Supporting research	0 - 10	(7.86)
Shelf life	0 - 10	(7.64)
Product range	1 - 10	(6.43)

Table 2. ***Ranking of desirable features of algal concentrates by hatcheries.***

Table 2 shows the results of part of a survey conducted for Western Biotechnology Ltd of Australian hatcheries where the respondents were asked to rank the important features of a concentrated microalgal product on a scale of 1 (little interest) to 10 (extremely desirable). Not surprisingly, the hatcheries rated nutritional value as most important, closely followed by consistency of supply.

Several ways of preserving concentrated algal biomass have been studied and these include dried biomass, frozen biomass and algal pastes or slurries. The use of dried or frozen algal biomass has been studied for many years. For example, Hidu and Ukeles (1962) used freeze-dried algae to feed the larvae of the clam, *Mercenaria mercenaria,* and frozen *Skeletonema* (Mock & Murphy, 1970; Mock, 1972) and *Tetraselmis* (AQUACOP, 1977) have been used in penaeid prawn culture. However, Brown (1972) found that frozen and freeze-dried *Skeletonema* and *Thalassiosira* were not as good as live diatoms as feed for *Penaeus aztecus*. Sun-dried *Chaetoceros* and *Tetraselmis* have also been used successfully to feed peneid larvae (Millamena *et al.*, 1990). Spray-dried heterotrophically grown algae have been available at various times (e.g. Cell Systems Ltd (UK) sold spray-dried *Tetraselmis suecica* and *Cyclotella cryptica* for about \$170.kg^{-1}). Beidenbach et al. (1990) showed that as much as 75% of live algae could be relaced by spray-dried *Tetraselmis* in the culture of *P. vannamei.* However these algae are generally nutritionally inadequate and their use has caused problems with fouling of the water (Laing *et al.*, 1990; Laing & Verdugo, 1991; Numaguchi & Nell, 1991; Laing & Millican, 1992; Curatolo *et al.*, 1993).

Wet algal concentrates (pastes or slurries) appear to be a much better alternative, and hatcheries in Canada and the USA produce limited quantities of these at various times. Algal concentrates have been used successfully in a number of research facilities and hatcheries (Watson *et al.*, 1986; Nell & O'Connor, 1991; O'Connor & Nell, 1992). Some algae such as *Tetraselmis* can be stored as pastes for very long periods (Montaini *et al.*, 1995) but long term storage while maintaining the nutritional quality of the algae has not yet been achieved for most species. Further work is essential therefore to extend the storage life of the algal concentrates and to develop appropriate methods of supplying these concentrates to the target species. The storage life (defined as the maximum time the paste/slurry can be kept and still retain a nutritional value equivalent to 'fresh' algae) ranges from about 1 week to 4-5 weeks, depending upon the species of alga. Wet pastes seem to be nutritionally better than then dried algae in part since they appear to maintain their original composition better. For example, Brown (1995) found that a wet paste of *C. calcitrans* stored at 4°C lost only 29% of its ascorbic acid content compared to algae dried at 60°C overnight which lost >94%. Freeze dried *C. calcitrans* also maintained its ascorbic acid content in storage, however >85% of the ascorbic acid was lost from the cells on resuspension. Similar degradation of cell contents, especially vitamins and carotenoids, upon freeze-drying and drum drying have been observed with other algae such as *Scenedesmus* and *Dunaliella* (Venkataraman & Becker, 1985; Ben-Amotz & Avron, 1989). These results may explain why frozen and dried algae are of lower nutritional value than fresh algae (Laing *et al.*, 1990).

Algal concentrates are not only a source of feed, but they have other potential advantages. Chemical and microbial loads associated with direct feeding of algal cultures have been found deleterious to some mollusc (Watson *et al.*, 1986) and crustacean (Zein-Eldin in Griffith *et al.*, 1973) larvae. Exclusion of growth media via the use of algal concentrates may therefore explain reports of enhanced larval growth and survival of some species when fed such concentrates (Nell & O'Connor, 1991). Accordingly, wider use of concentrates could facilitate production of species for which efficient output of consistently high quality postlarvae or juveniles has thus far proven elusive. Such species include the commercial scallop, *Pecten fumatus* (M. Heasman - Pers. Comm.); the Jumbo tiger prawn, *Penaeus monodon*; the Sydney rock oyster, *Saccostrea commercialis* (Nell *et al.*, 1991) and the silverlip pearl oyster, *Pinctada maxima* (R. Rose - Pers. Comm.).

The availability of low cost algal concentrates would also allow the blending of these concentrates to produce nutritionally superior feeds. Not surprisingly, unialgal diets are generally not as nutritionally advantageous as mixed algal (or algae and yeast) diets (Epifanio, 1979b; Gallardo *et al.*, 1995). Although many hatcheries do produce mixed diets, the scope for this is limited due to the cost of maintaining a range of algal species.

METHODS

Algae and Medium

The algae used in this study were: *Tetraselmis chuii, Tetraselmis suecica, Isochrysis galbana, Pavlova lutheri, Chroomonas salina, Skeletonema costatum, Chaetoceros gracilis* and *Chaetoceros calcitrans*. The algae were obtained from the CSIRO microalgal culture collection, Hobart, Tasmania.

The medium used for all cultures in this study was based on the *F/2* medium of Guillard and Ryther (1962) (Table 3). For laboratory cultures the sea water was filtered through a Whatman carbon cap activated charcoal filter. For the large BIOCOILs the activated carbon filtration step was omitted and the sea water was filtered through a series of filters to 0.2 μm It was then diluted 25% with deionised water (laboratory cultures) or tapwater (large-scale cultures).

For laboratory cultures, stock nutrient solutions, with the exception of phosphate, iron and vitamins, were added at the concentrations shown in Table 1 and the pH adjusted to pH 7.5 with 1M HCl. The medium was then autoclaved at 109°C at 5 psi for 45 minutes. Iron and phosphate stock solutions were autoclaved separately to avoid precipitation. These solutions, along with the vitamin stock solutions, were added aseptically at room temperature in a laminar flow hood by filtration using 0.2 μm membrane filters. (In some experiments the seawater was sterilised in situ by adding 1.25 $mL.L^{-1}$ of 12.5% sodium hypochlorite and mixing the culture for at least 1h. The hypochlorite was then neutralised by the addition of 100 $mg.L^{-1}$ of sodium thiosulphate.).

For the large-scale cultures the nutrients were sterilised separately and then added aseptically to the reactors.

Sterility checks

The sterility of the cultures was checked regularly by plating 100 μL of culture on R2A medium (Table 4) solidified with 1.5 $g.L^{-1}$ agar. The plates were incubated at 28° C in the dark and any cultures showing growth after 2 days were discarded. This method was also used for determining bacterial load in the photobioreactors.

Stock cultures and inoculum preparation

Algal stock cultures were maintained with regular subculturing in 30 mL of *F/2* medium in 50 mL Schott bottles at a constant temperature of 23 °C and an irradiance of approximately 100 μmol photons.m^{-2}.sec^{-1} supplied by 18 W cool-white fluorescent tubes. Experimental inocula were prepared by inoculating 300 mL of *F/2* medium in 500 mL Schott bottles held under the same conditions. These cultures were then used to inoculate 4 L of *F/2* medium in sterile 5 L or 20L glass carboys fitted with air spargers and stirring bars. These were kept under the same conditions above except for aeration and stirring. The 5 L carboy cultures where then used to inoculate the bioreactors.

Table 3: ***The composition of stock solutions and final concentration in F/2 medium***

Stocks	Stock Solution ($mg.100\ mL^{-1}$)	Volume added ($mL.L^{-1}$)	Final concentration ($mg.L^{-1}$)
$NaNO_3$	--	--	150.0
Ferric Citrate	9.0	1	90.0
$NaH_2PO_4.2H_2O$	1.0	1	10.0
$NaSiO_3.9H_2O$	0.5	1	5.0
$MnCl_2.4H_2O$	0.036	1	0.36
$CoCl_2.6H_2O$	0.002	1	0.02
$ZnS0_4.7H_2O$	0.004	1	0.044
$CuSO_4.5H_2O$	0.00196	1	0.0196
$NaMoO_4.2H_2O$	0.0013	1	0.013
Vitamin mix:			
Thiamine	20.0	1	0.2
Biotin	1.0		0.01
Cyanocobalamin	1.0		0.01

Table 4: ***The composition of R2A medium used for algal sterility checks***

Ingredients	Concentration ($g.L^{-1}$)
Yeast extract	0.5
Difco Bactopeptone	0.5
Glucose	0.5
Soluble starch	0.5
Sodium acetate	0.3
K_2HPO_4	0.3
$MgSO_4.7H_20$	0.05

Photobioreactor setup and inoculation

When new, all photobioreactors were initially treated by filling with acidified water and circulating this for 24h. This treatment was repeated at leat 3 times to ensure leaching of plasticisers and other compounds which can affect algal growth.

Before an experiment the photobioreactors reactors were sterilised by filling with water containing 0.2 $mL.L^{-1}$ of 12.5% (v/v) sodium hypochlorite. After addition of the sodium hypochlorite the medium was pumped for approximately 24h. In the pilot-scale units it was found necessary to repeat this treatment at least once. The reactor was then washed with 0.2 µm filtered water before addition of 0.2 µm filtered seawater. The medium was again pumped for 30 min and then left to sit for 24 h. F/2 medium nutrients were added aseptically and the reactor inoculated.

Photosynthesis Measurements

A Rank Brothers (UK) polarographic Clark-type oxygen electrode was used to measure photosynthesis and respiration in four algal species: *T. chuii, T. suecica, I. galbana and, S. costatum*. The temperature in the water jacketed glass electrode chamber was controlled by a controlled temperature water bath and light was supplied to the chamber by a slide projector fitted with a quartz halogen bulb. The electrode was linked via a Rank Brothers oxygen meter to a chart recorder. The electrode was calibrated at each temperature to zero O_2 by passing ultra pure nitrogen gas over the membrane surface for approximately 5 min. The electrode was calibrated to 100% O_2 saturation by filling with air-saturated tap water. Irradiance (PAR) was measured at the surface of the chamber with a Li-Cor model LI-185B Quantum meter. O_2 concentration in the saturated water was calculated using the tables of Carpenter (1966).

Oxygen concentration effect study

The effect of oxygen concentration on the gross photosynthetic rate was investigated by allowing the oxygen concentration within the chamber to increase from >10% to 150% saturation in continuous light. The culture was then removed for chlorophyll analysis. The above method was repeated twice for each of the three temperatures at both irradiances tested. pH was measured before and after each run to check whether the cultures might be CO_2 limited. Control experiments where sodium bicarbonate was added to the chamber were also carried out.

Cell counts

The cell densities were measured using a standard haemocytometer. Due to high motility, samples of *Tetraselmis* and *Isochrysis* were treated with 4 drops of "Lugols" solution (0.07 g of iodine and 60 g of KI per 50 mL plus five drops of 1M NaOH) prior to pipetting.

Dry Weight

Dry weights were determined by filtering a measured volume of culture (5 to 15 mL) onto a pre-dried, pre-weighed Whatman GF/C 2.5 cm diameter glass fibre filter. Pre-dried filters were stored in a vacuum desiccator prior to use after they had been washed by soaking in deionised water for one hour and dried overnight at 100 °C. Samples were filtered till dry and washed twice with 10 mL of 0.65M isotonic ammonium formate. The samples were then placed in an oven and dried at 100 °C for one hour before being transferred to a vacuum desiccator overnight. Both pre-weighing and final weighing of filters was done to four decimal places. Dry weight ($g.L^{-1}$) was determined by subtraction of the filter weight from the filter + dried sample weight divided by the volume filtered.

Chlorophyll

Chlorophyll determination was carried out using a modified 90% acetone extraction based on Jeffrey & Humphrey (1975).

A measured volume of culture (5 to 15 mL) was filtered onto a Whatman GF/C 2.5 cm diameter glass fibre filter and washed twice with 10 mL of 0.65M isotonic ammonium formate. The filter was then homogenised in 10 mL of 90% acetone (v/v) in a hand held glass homogeniser chilled

in ice. The homogenate along with approximately 2 mL of washing's (90% acetone) was then transferred to a 15 mL graduated glass centrifuge wrapped in foil, stoppered and then stored at 4°C overnight in the dark to complete extraction. After centrifugation for 5 min the sample volume was recorded. The absorbance was measured using quartz cuvettes against a 90 % acetone blank in a Hitachi U-1100 spectrophotometer. The chlorophyll content ($\mu g.mL^{-1}$) was then calculated using the equations of Jeffrey & Humphrey (1975):

Photobioreactor Design

The photobioreactor used in this work was a helical tubular photobioreactor, the BIOCOIL®. The basic principles and design of this reactor are patented (Robinson *et al.*, 1988; Robinson & Morrison, 1992). The BIOCOIL, as used in this study, consists of a photostage constructed of 24mm i.d. clear food-grade PVC tubing wrapped around a cylindrical support made of wire mesh supported by a steel framework. The algal culture is circulated through this tubing by means of a pump. For 'tough', shear-tolerant algae such as *Chlorella* and *Spirulina* this pump may be a centrifugal pump, a diaphragm pump, a screw pump or a lobe pump. However, the algal species used in aquaculture are generally more delicate and are damaged by shear forces generated in these pumps so we developed and tested several airlift designs which generated the necessary flow rates without causing significant cell damage. Since the system is a 'closed' reactor there is also the need to remove excess O_2 produced by algal photosynthesis and to supply CO_2. The airlift serves this purpose, however a gas exchange tower can also be added.

The basic design of the laboratory-scale units (approx. 40 - 70L in volume depending on configuration) is shown in **Figure 3**. The photostage of these units consisted of a single length of tubing (39.5m in length for BR1 and 79m in length for BR3) with the connections between the airlift and the photostage modified so that the direction of flow within the photostage could be reversed (see **Figure 3**). All fittings (valves, connectors etc.) were non-metal to avoid possible contamination of the cultures. Air for the airlift was supplied by a small air pump and passed through a 0.2 μm filter before entering the airlift. Throughout the study we continually improved the design of the airlifts to give better flow rates and smaller bubble size (smaller bubble size reduced damage to the algal cells). This was found to be very important for the successful culture of various algal species. Flow rates were maintained at about 30-40 $cm.sec^{-1}$; this rate is important to keep the algal cells in suspension at all times and to reduce adhesion of the algal cells to the inside of the photostage tubing.

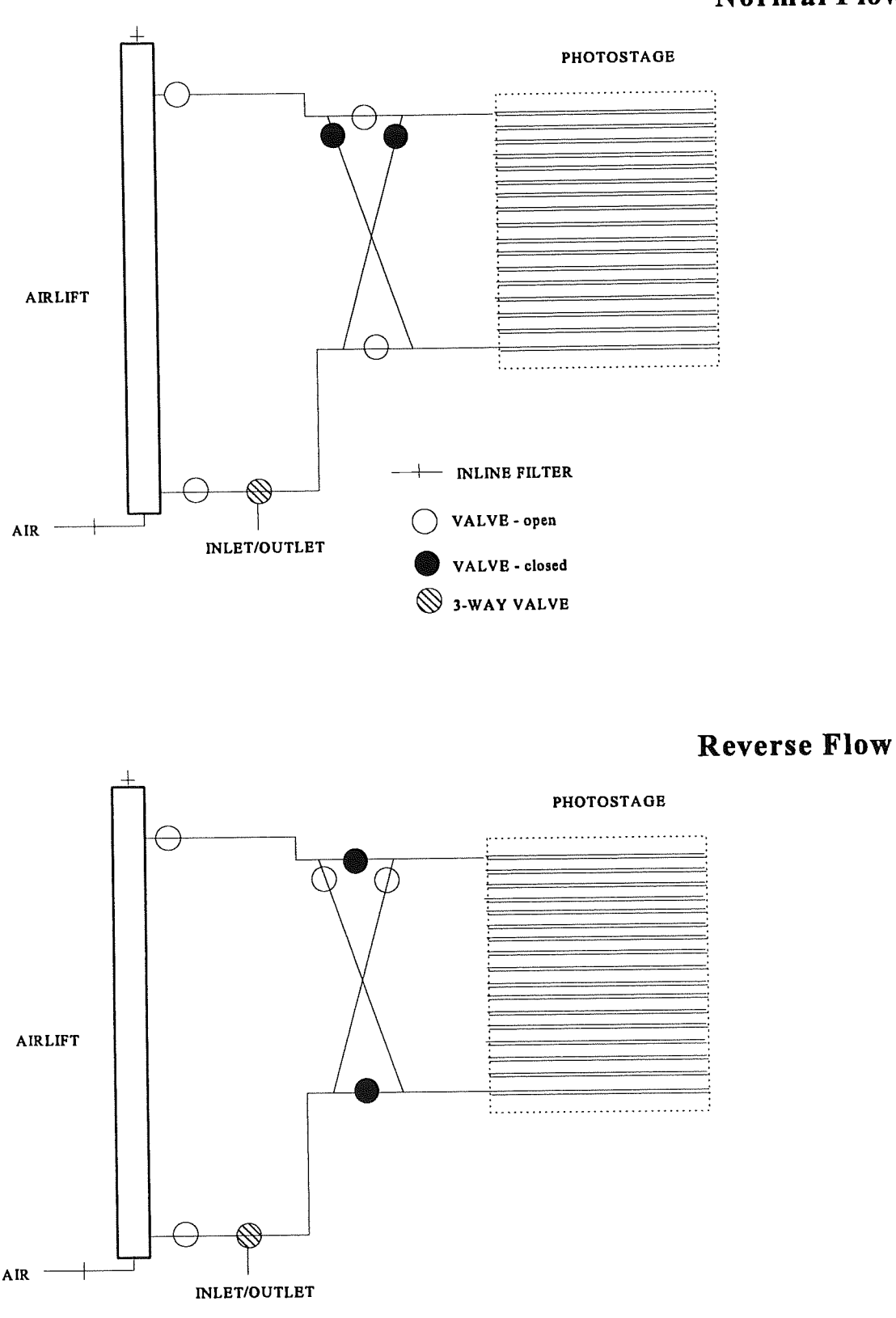

Figure 3 Schematic diagram of the laboratory BIOCOILs (approx 30 - 70L in volume) showing configuration during normal flow (top) and reverse flow (bottom). In some configurations a heat exchange tower (not shown) was also located between the airlift and the photostage.

Temperature control was achieved either by a heat exchanger located in a glass tower between the photostage and the airlift (this is not shown in **Figure 3**) when the reactors were located in the laboratory or glasshouse, or by placing the reactor in a temperature controlled room.

Illumination was supplied by fluorescent tubes (mixture of cool white and Growlux) placed within the photostage tower as well as around the outside of the photostage and giving an irradiance of about 50 - 100 μmol photons.m^{-2}.sec^{-1} at the reactor surface. In some experiments a quartz-halogen lamp was used to provide an irradiance of about 2200 μmol photons.m^{-2}.sec^{-1} on one side of the reactor.

The design of the two 550L (average volume - they could be increased to 680L is required) pilot-scale BIOCOILs (**Figure 5**) was based on experience with the laboratory-scale reactors. These pilot reactors were located outdoors in Perth with daylight as the only form of illumination.

The photostage consisted of 15 parallel 'wraps', each of 45m of 24mm i.d. PVC tubing (ie. the same tubing as in the laboratory BIOCOILs), wound around a cylindrical steel mesh frame (**Figure 6**). The parallel arrangement of the 'wraps' is an important feature of the BIOCOIL design which allows more even flow through the tubing and a shorter tube length per 'pass' of the algae through the tubing thus minimising O_2 build-up in the tubing. The individual 'wraps' of tubing are connected to an inlet and an outlet manifold and these manifolds are connected to the airlift. Each tubing connection to the manifold was fitted with a ball valve (**Figure 7**) so that any particular 'wrap' could be isolated from the rest of the system. This was important when cultures were first inoculated as the total volume of the reactor could be reduced by connecting only one or a few 'wraps'. The reactor could then be operated at this smaller volume until the culture had reached a sufficiently high density to increase the volume by adding more 'wraps' (i.e. part of the inoculum could be cultured *in situ*). The photograph in **Figure 6** shows the reactor with the top 'wrap' without algal culture.

These 550L reactors also used an airlift with air supplied by a large compressor which could supply up to 120 L air per minute. The air passed through an oil filter and a pre-filter before being filtered to 0.2 μm with a cartridge filter (Gelman). Flow rates could be adjusted by adjusting air flow (**Figure 8**). Experience has shown that flow rates need to be between 30 and 40 cm.min^{-1} to prevent settling of the cells and to generate enough turbulence (high Reynolds number) to minimise sticking of the cells to the tube walls.

The air outlet for the reactors was located at the top of the header box between the airlift and the outlet manifold and consisted of a long downward facing PVC pipe. After some initial contamination problems this pipe was arranged so that it discharged into a bath of acidified (pH <2) water to prevent entry of contaminating organisms via this route.

All piping between the water filters and the reactor were arranged so that they could also be sterilised *in situ* with hypochlorite.

As with the laboratory BIOCOILs, no metal parts were in contact with the algal culture.

Temperature control in these units was by evaporative cooling this was achieved by running water over the surface of the photostage when the culture temperature exceeded a given set point. The

water was collected in a sump at the base of the photostage from which it was pumped over the photostage surface.

Sterilization of both the laboratory and the pilot reactors was by filling the reactors with water and adding sodium hypochlorite to give a final 1% w/v concentration. In the laboratory reactors the medium was sterilized either by hypochlorite treatment or by autoclaving and then adding it aseptically . Both methods worked well and no difference in algal growth was observed. The pilot reactors were sterilised in the same way by filling them with water, adding hypochlorite and then circulating for at least 12h. The process was then repeated at least once. Seawater for the medium was sterilised by filtering to 0.2 μm using a series of autoclavable in-line filters (Pall).

After each run the inside of the reactors was cleaned by first flushing with fresh water and then with water plus hypochlorite. This was generally adequate to clean the inside of the tubes, however after very long culture periods we found that this cleaning process had to be repeated several times. We therefore developed a cleaning method using high pressure air in addition to the washing procedure.

When CO_2 was added to the reactors this was done on a pH-stat method; i.e. pH was kept constant by the addition of CO_2 as required. A pH probe was mounted in-line on the outlet side of the photostage and, when the pH exceeded a set point, pure CO_2 was added to the airlift air stream until the pH fell below the set point. At various times the reactors were also fitted with a O_2 probe.

Table 5 summarises some of the characteristics of the basic laboratory BIOCOIL (type BR1) and the pilot scale BIOCOILs.

Table 5. Comparison of basic characteristics of a laboratory-scale BIOCOIL and a pilot-scale BIOCOIL.

	Laboratory BIOCOIL (BR1)	Pilot-scale BIOCOIL
Volume (L)	35-40	approx 550 (up to 680)
Height (m)	1	2.5
Diameter of photostage (m)	0.6	2.2
Number of 'wraps'	1	15
Total tube length (m)	39.5	675
Photostage surface area (m^2)	0.988	15
Lit volume (L)	19.4	331
Lit volume (%)	46	64
Air lift height (m)	1.2	2.7
Airlift i.d. (cm)	5.5	14
Aspect ratio	22	21
Bubble size in airlift (mm)	> 4	< 4
Flow rate ($cm.sec^{-1}$)	25 - 50	20 - 40

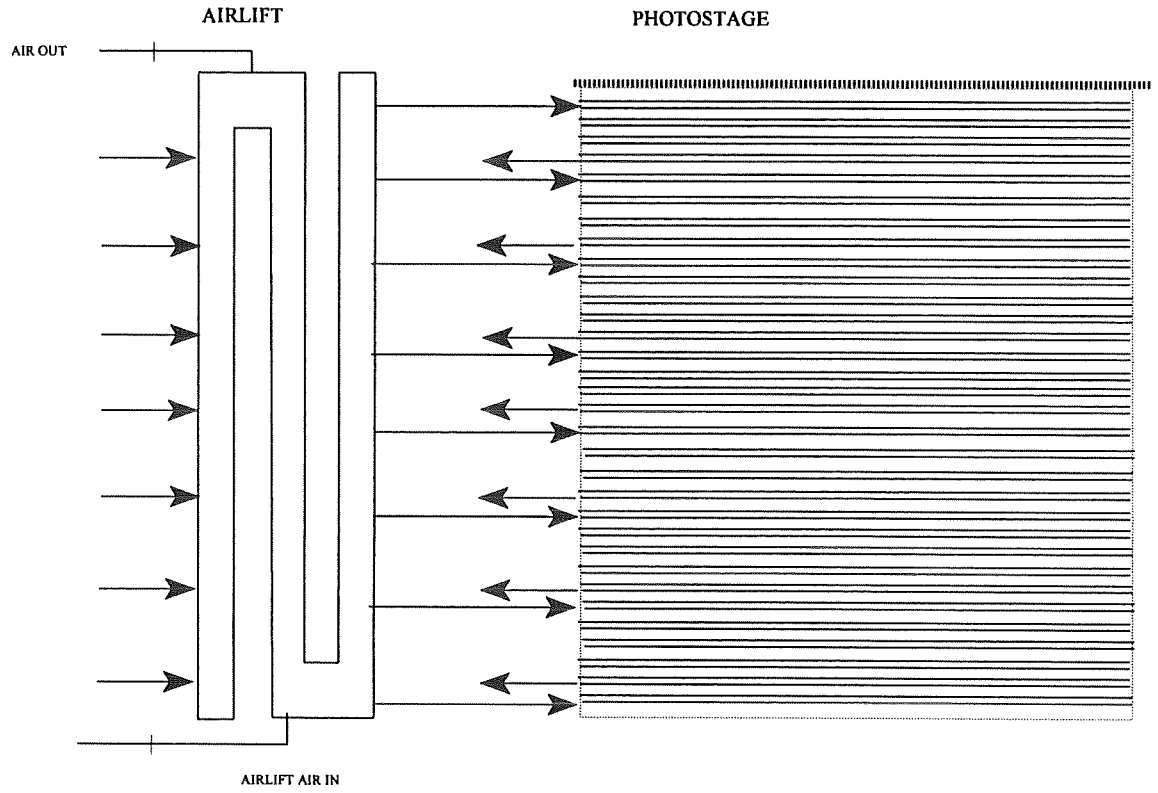

Figure 5 Schematic diagram of 550L pilot-scale BIOCOIL. The arrows indicate flow into or out of the photostage (for clarity connections are not shown). For clarity this diagram shows only 7 'wraps'; the pilot-scale units actually have 15 'wraps' (see **Figure 6**). Each wrap can be isolated from the manifolds by ball valves (not shown) located at the inlet and outlet manifolds (see **Figure 7**). On the top of the photostage tower there is a perforated pipe (Dark dotted line) which flows water over the photostage for cooling.

Figure 4 Photograph of 550L pilot-scale BIOCOIL located at Murdoch University, Perth, showing the photostage. The airlift is located behind the photostage. The BIOCOIL is mounted on a base which acts as the reservoir for the cooling water. The diameter of these units is less than optimum so that the units are easily transportable. Note the absence of culture in the top 'wrap' of the photostage tower.

Figure 5 Photograph showing tubes of the 'wraps' entering the inlet manifold on the pilot-scale Biocoils.

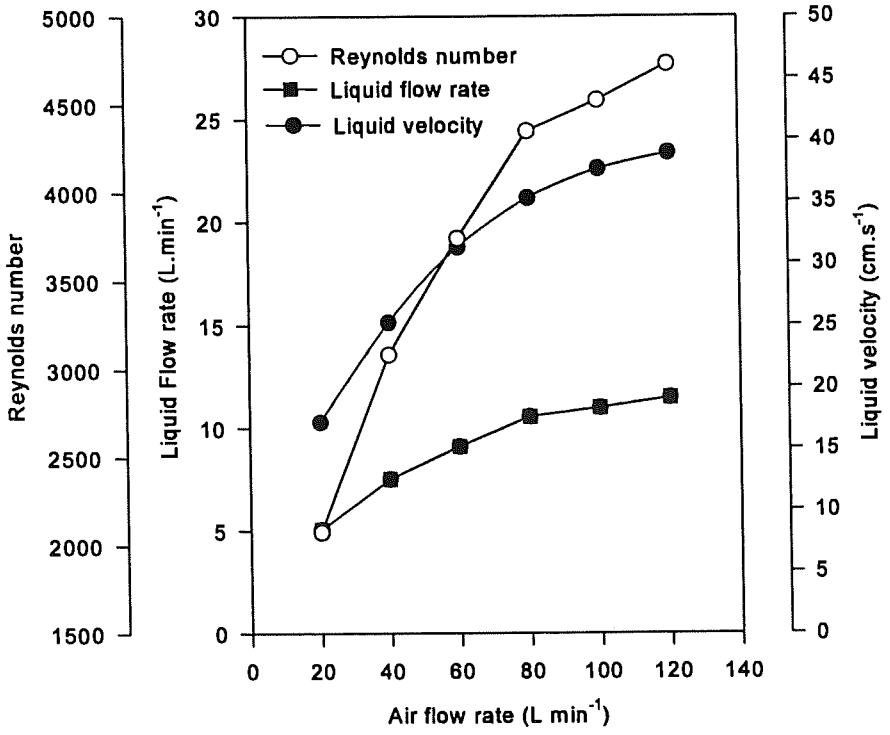

Figure 8 Relationship between air flow rate and liquid velocity, liquid flow rate and Reynolds number in the 550L pilot-scale BIOCOILs.

RESULTS

Optimisation of growth conditions

One of the important factors for lower cost large-scale culture of microalgae for aquaculture is the ability to use natural daylight rather than artificial lighting. However, outdoor culture also means that temperature control is more difficult and algae with higher temperature tolerances are preferred. Preliminary experiments were carried out to examine the effects of temperature on several of the species in order to determine whether the species could be cultured at about 23-26°C outdoors or whether the bioreactors had to be chilled to lower temperatures. Theoretical calculations indicated that it would probably not be possible to maintain the reactor temperature at less than about 23°C with evaporative cooling only.

For some species we also examined whether additional nutrients such as vitamins or silicon were required for optimal growth.

Finally, we also examined the effect of day length, since outdoor cultures would not normally be exposed to continuous light. All these experiments were carried out in 50ml culture flasks in either a controlled temperature growth room or in environmental cabinets at irradiances of 60 to 80 µmol photons.m^{-2}.sec^{-1}.

Temperature

The effect of culture temperature was examined for *Tetraselmis suecica*, *T. chuii*, *Chaetoceros calcitrans*, *C. gracilis*, *Isochrysis galbana* and *Pavlova lutheri*.

The growth rate and final cell yield of both the *Tetraselmis* species was not significantly different between 16°C and 24°C (**Figure 9** and **Figure 10**). Similarly, growth of *C. calcitrans* was not significantly affected over the same temperature range (**Figure 11**). On the other hand, *C. gracilis* grew better at higher temperatures with the fastest growth at 28-32°C (**Figure 12** and **Figure 13**). *Isochrysis galbana* grew faster at 24°C than at 15°C (**Figure 15**) whereas *P. lutheri* was little influence over the temperature range tested (**Figure 14**).

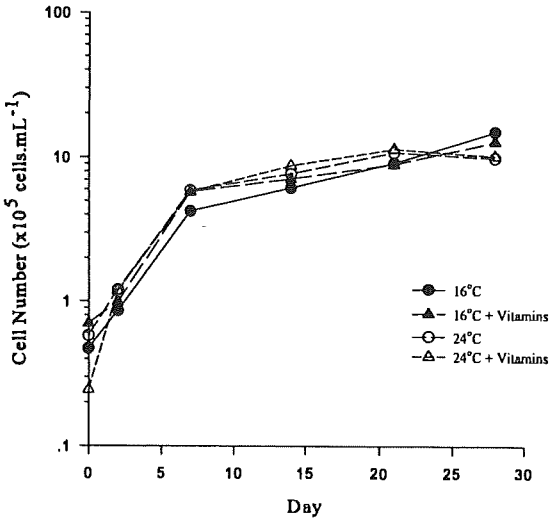

Figure 9 *Tetraselmis chuii*. Growth at various temperatures and with Vitamins added (T1-46).

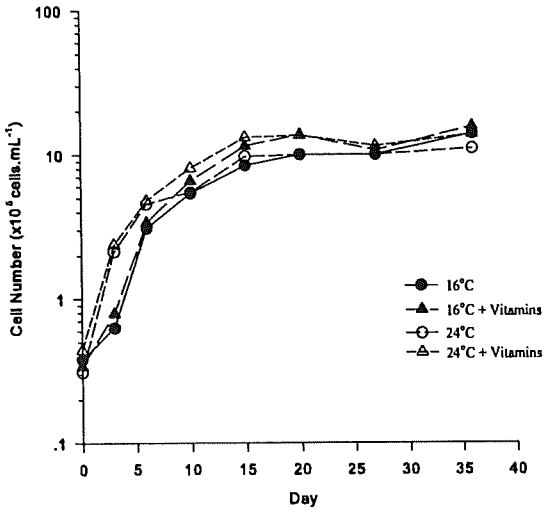

Figure 10 *Tetraselmis suecica.* Effect of temperature and vitamins on growth (T1-41).

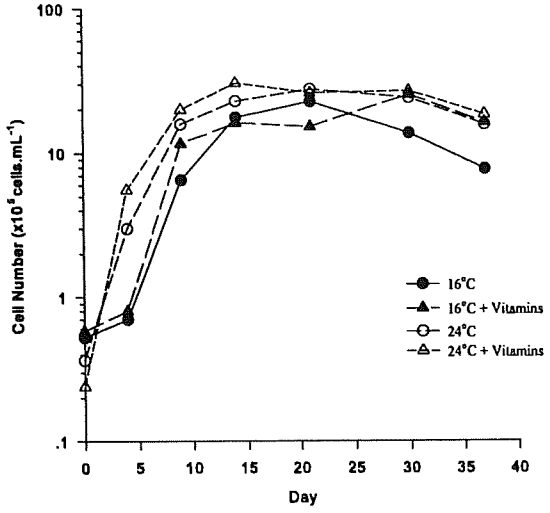

Figure 11 *Chaetoceros calcitrans.* Effect of temperature and vitamins on growth (T1-43).

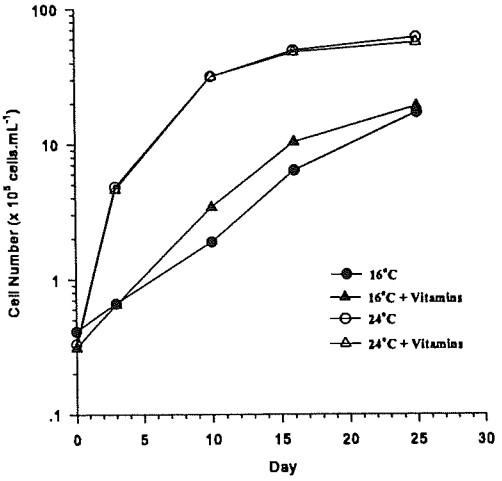

Figure 12 *Chaetoceros gracilis.* Effect of temperature and vitamins on growth (T1-59).

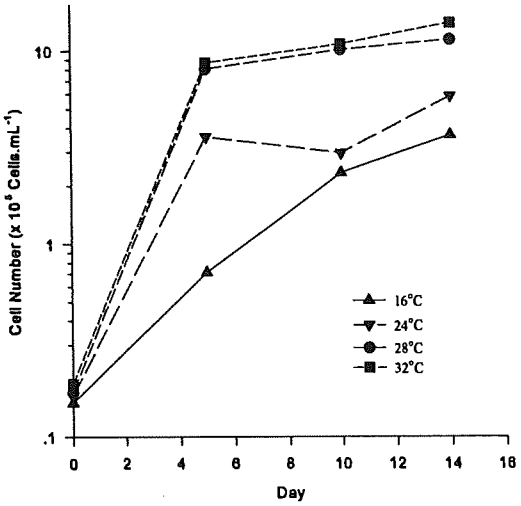

Figure 13 *Chaetoceros gracilis.* Effect of temperature on growth [no vitamins were added] (T1-64).

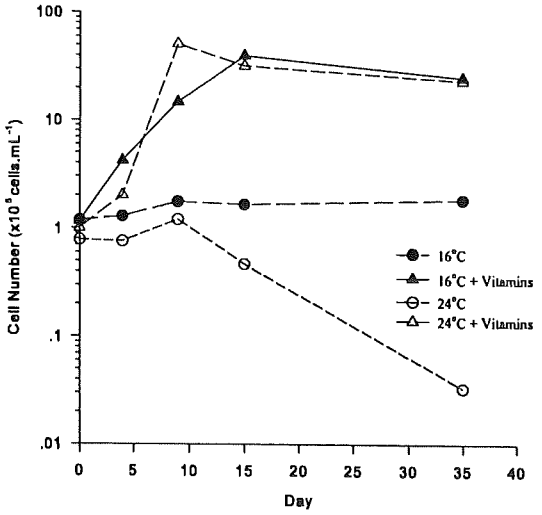

Figure 14 *Pavlova lutheri.* Effect of temperature and Vitamins (T1-52).

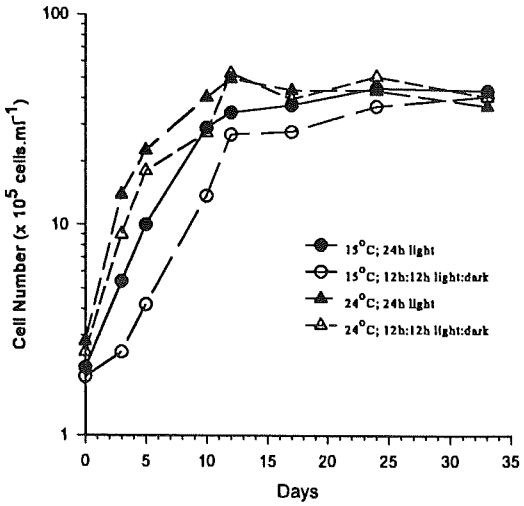

Figure 15 *Isochrysis galbana* (T-iso). Effect of temperature and day length (T1-38).

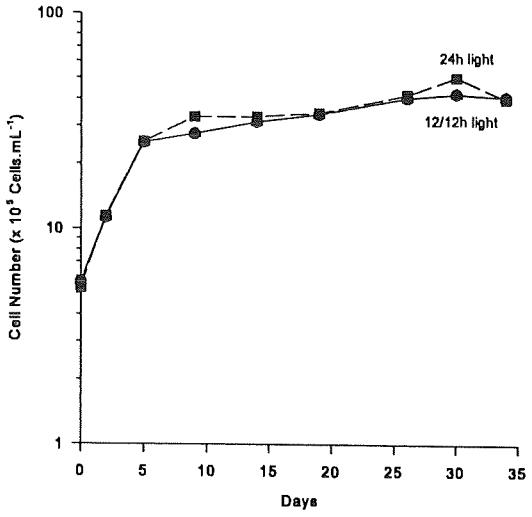

Figure 16 *Pavlova lutheri.* Effect of day length (T1-36).

Vitamins

Some algae require vitamins for optimum growth, however in large-scale systems the addition of vitamins adds another cost factor. We therefore examined the effect of vitamin supplementation to the medium at the same time as doing the above temperature experiments.

The addition of vitamins had no significant effect on the growth rate and cell yield of the *Tetraselmis* species (**Figure 9** and **Figure 10**) nor on the *Chaetoceros* species (**Figure 11** and **Figure 12**). *Pavlova lutheri*, however, does have a clear vitamin requirement (**Figure 14**). For any significant growth to be achieved vitamins had to be added. As well as this, cell numbers in the 24°C culture without vitamins declined rapidly after about 10 days.

Day length

Indoors cultures can be grown in 24h light, however this is not economically feasible outdoors. Two of the species were tested in the laboratory to examine the effect of a 12h light:12h dark cycle as compared to 24h light. *Isochrysis galbana* showed some reduced growth under the light :dark cycle, however the final cell density reached after 24 days was the same (**Figure 15**). *Pavlova lutheri* was unaffected by being frown in continuous light or under a light:dark cycle (**Figure 16**).

Growth in laboratory-scale tubular bioreactors

All algae were initially grown in 30-60L BIOCOILs, indoors under artificial lighting. Initial cultures were in batch mode, and in longer term experiments the cultures were operated in semi-continuous mode. Some cultures were later carried out in the same bioreactors, but in a glasshouse under ambient irradiances. The aims of the experiments were:

- To establish that the various algal species could be gown in the BIOCOIL
- To develop a culture management regime for long-term culture of the algae
- To improve culture conditions and the design of the BIOCOIL in order to increase productivity, cell density and culture stability
- To determine what factors were limiting algal productivity and cell density

Tetraselmis suecica & Tetraselmis chuii

Tetraselmis suecica was first grown in the laboratory in a small BIOCOIL in two stages: (1) in the clear tower used for temperature control in order to increase cell density before adding the (2) photostage of the BIOCOIL into the circuit.

Figure 17 shows the results of this experiment. The algae grew well in the tower but grew slower once the photostage had been incorporated in the circuit. After about 13 days in the complete BIOCOIL, cell numbers appeared to decline and this decline could not be arrested by addition of fresh nutrients. A significant component of this apparent decline in cell numbers was due to adhesion of the cells to the walls of the culture vessel. Although most of the sticking cells could be dislodged by vigorous beating of the tubing of the photostage, they quickly re-adhered. The flow rate in this reactor was about 20 cm.sec^{-1}. In order to achieve a higher flow rate and Reynolds number which should reduce the adhesion of the cells the airlift was redesigned for later experiments.

Growth in the modified BIOCOIL was better with less cell adhesion. Placing the BIOCOIL in a glasshouse where the irradiance during the day was greater also increased the maximum cell density reached (**Figure 18**). This was the first experiment outdoors in a glasshouse and temperature control proved to be a problem. The cooling system used proved

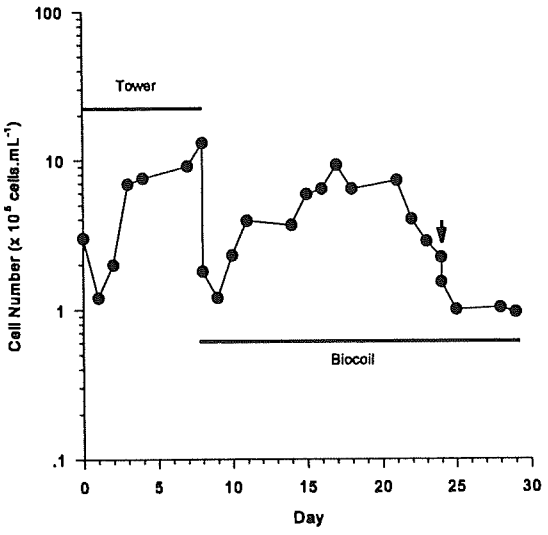

Figure 17 *Tetraselmis suecica* initially cultured in the heat-exchange tower of the BIOCOIL before including the photostage. Final volume 38L. Arrow = addition of extra f/2 nutrients. [Reactor BR1 in laboratory, temp 22±1°C][T1-42b]

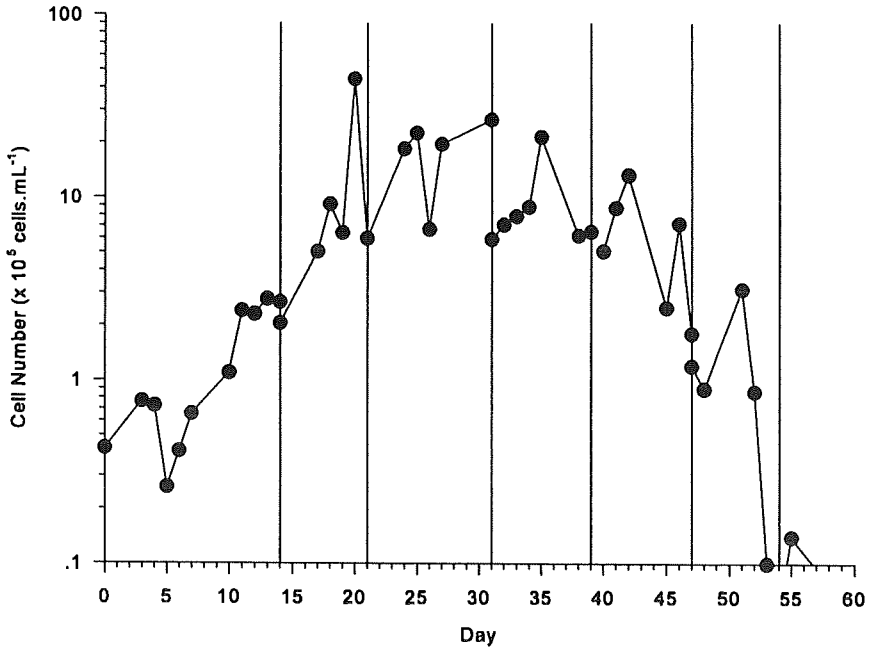

Figure 18 *Tetraselmis suecica.* Growth in 38L BIOCOIL in glasshouse under ambient light supplemented with fluorescent lights at night. Temperature control by means of heat exchanger (cooling only). Vertical lines = partial 12L change of medium. [T1-47].

inadequate on hot days and temperatures exceeded 28°C in the reactor and this is presumed to be the reason for the decline in the culture after about 40 days growth and the eventual death of the culture (**Figure 18**).

The experiments with *T. suecica* were continued with *T. chuii*, a species of *Tetraselmis* which preliminary experiments had shown to stick less and to be less temperature sensitive than *T. suecica.* **Figure 19** shows the growth of *T. chuii* in a 38L BIOCOIL in the laboratory. This culture reached cell densities > 1 x 10^6 cells.mL^{-1} (= approx. 1.1 g dry weight.L^{-1}) (**Figure 19**).

When grown in the glass house in a BIOCOIL with improved temperature control the culture could be maintained in excess of 140 days (**Figure 20**). The mean maximum cell density in the glasshouse culture was about double that in the laboratory culture and this is attributed mainly to the higher irradiance in the glasshouse. The indoor irradiance was less than 200 μmol photons. m^{-2}.sec^{-1} whereas the noon irradiance outdoors was greater than 2000 μmol photons.m^{-2}.sec^{-1} even on cloudy days.

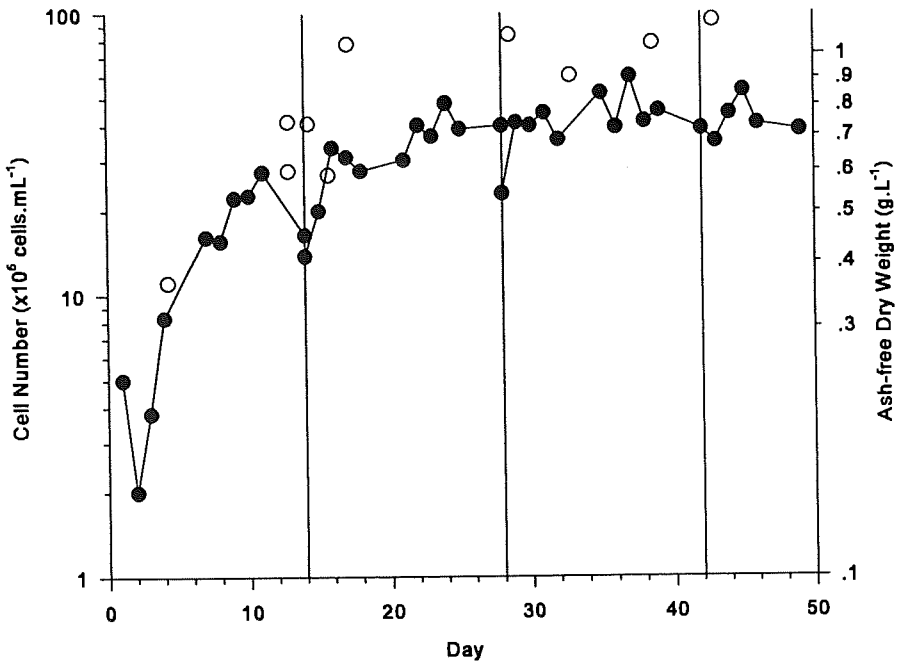

Figure 19 *Tetraselmis chuii*. Growth in 38L Bioreactor in laboratory showing both cell numbers (●) and ash-free dry weight (O). Vertical lines = partial medium change. [Temperature 22°C±1°C].

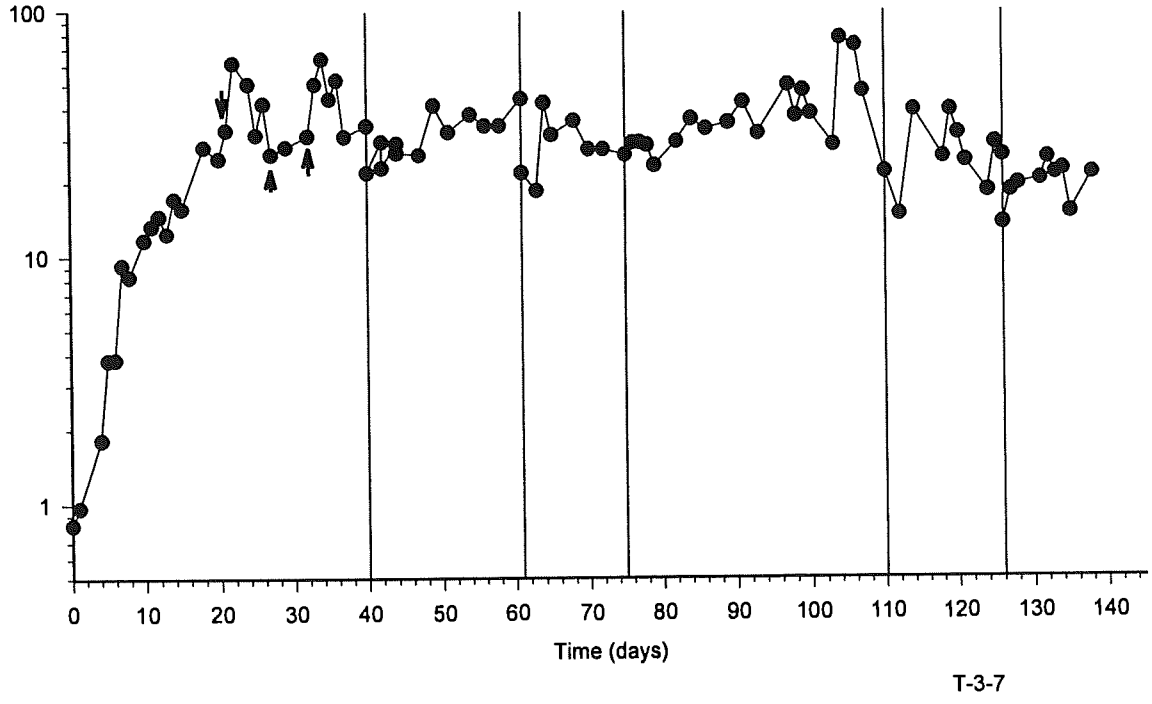

Figure 20 *Tetraselmis chuii*. Growth in 38L BIOCOIL (BR2) in glasshouse. Vertical lines = partial exchange of medium. Arrows = addition of N, P and Fe.

Isochrysis galbana (strain T-iso)

The first long-term culture of *I. galbana* indoors in the culture room resulted in good growth and biomass (**Figure 21**), however sticking of the cultures to the inside of the bioreactor tubes meant that cell counts were somewhat variable. By regular harvesting of part of the culture and replacing

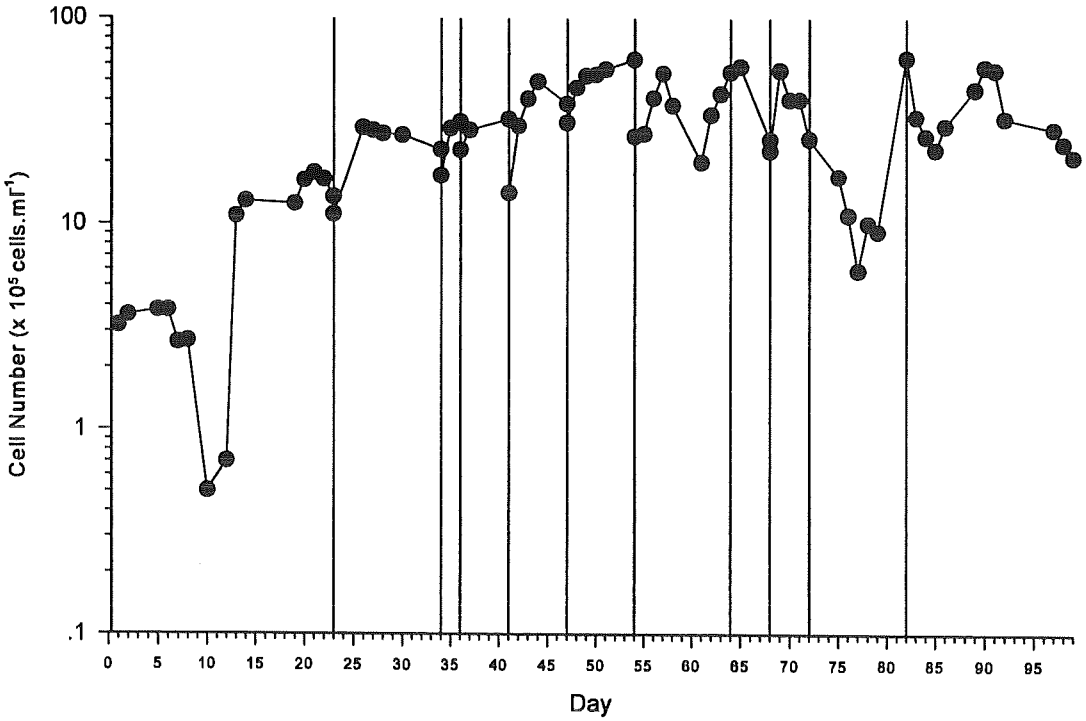

Figure 21 *Isochrysis galbana* (T-iso). Growth in 38L BIOCOIL (BR3) in laboratory. Vertical lines = partial change of medium. [Temperature 22-26°C]. NB The initial low growth and apparent decline on day 10 was due to a series of air pump failures.

the harvested volume with fresh medium (i.e. semi-continuous culture) continued good growth of the culture could be maintained. The culture was terminated after 98 days to permit other experiments to be undertaken.

When a similar growth experiment was carried out in a glasshouse in the same type of bioreactor, however with a pH-stat and with an additional evaporative cooling system added, very good growth and high cell densities were achieved (**Figure 22**). This high productivity could be maintained for many weeks with regular harvesting of cells and replacement with fresh medium, even at a high rate (see **Figure 22**). Some sticking of cells still occurred, but this was remedied by reversing the flow in the photostage periodically for about 30 min. The maximum cell density achieved outdoors was significantly higher than indoors, presumably due to the higher irradiance (compare **Figure 21** with **Figure 22**).

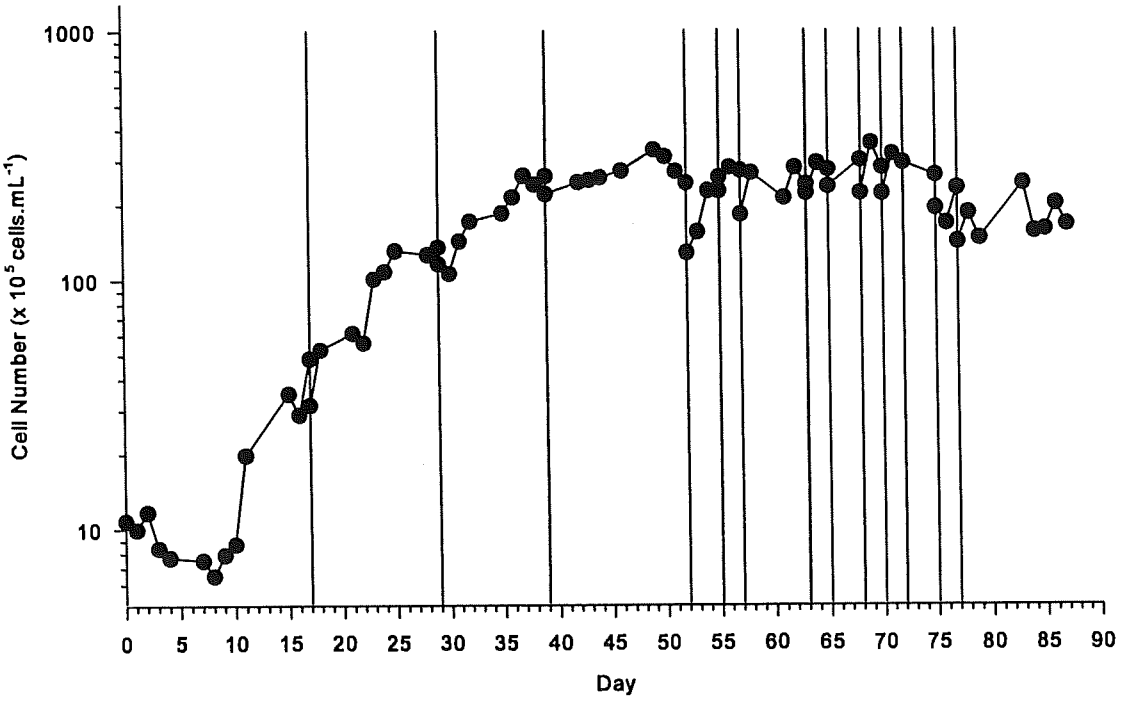

Figure 22 *Isochrysis galbana* (T-iso) in 38L BIOCOIL (BR2) in glasshouse with additional evaporative water cooling installed. During the period of no growth the cooling system was not operating reliably and the CO_2 pH-stat was also not functioning properly leading to excessive sticking of the algae to the inside of the photostage tubes. Vertical lines = partial harvest and medium change. [T1-55].

Pavlova lutheri

Figure 23 shows the growth of *Pavlova lutheri* in a laboratory BIOCOIL. Growth rate and cell yield were excellent and the culture was quite stable for the 42 days of the experiment with regular partial harvests and media changes. The experiment was terminated in day 42 due to a compressor breakdown.

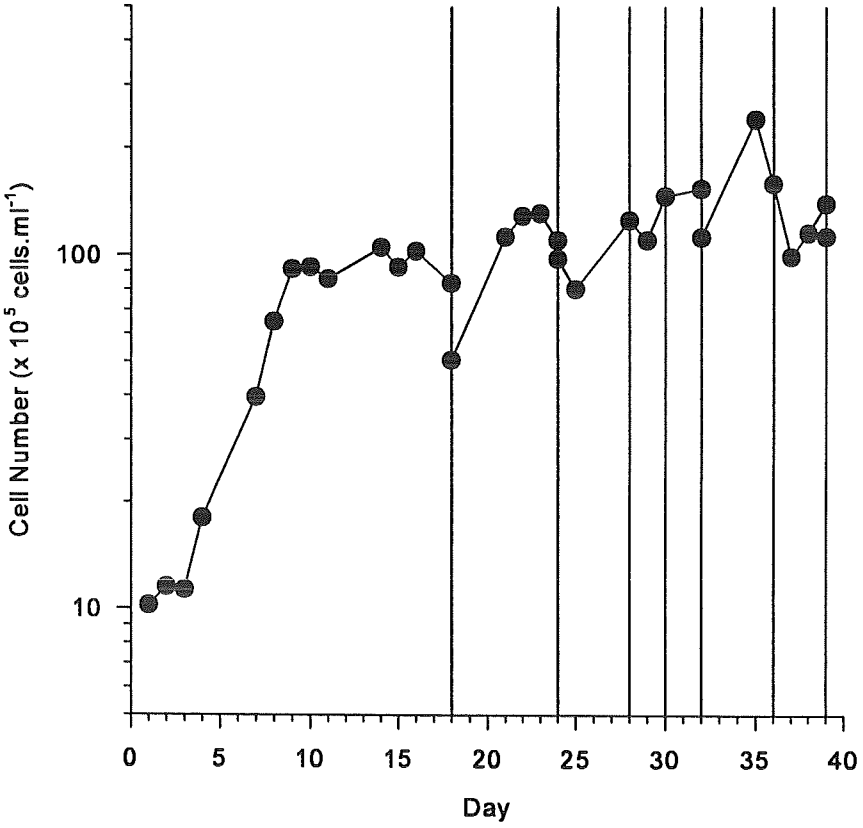

Figure 23 *Pavlova lutheri*. Growth in 38L laboratory BIOCOIL with CO_2 pH-stat. Temperature = 22±2°C [T1-61].

Skeletonema costatum

This diatom is well known to 'crash' soon after reaching the stationary growth phase in culture (Lee & Tamaru, 1993; Liao *et al.*, 1993a). In the first growth trial in a 38L BIOCOIL in the laboratory the culture grew well until day 6 and then declined rapidly (**Figure 24**). A 40% replacement with fresh medium on day 13 led to a renewed brief growth before the culture declined again. These results suggested that the decline in cell number was due to some nutrient deficiency.

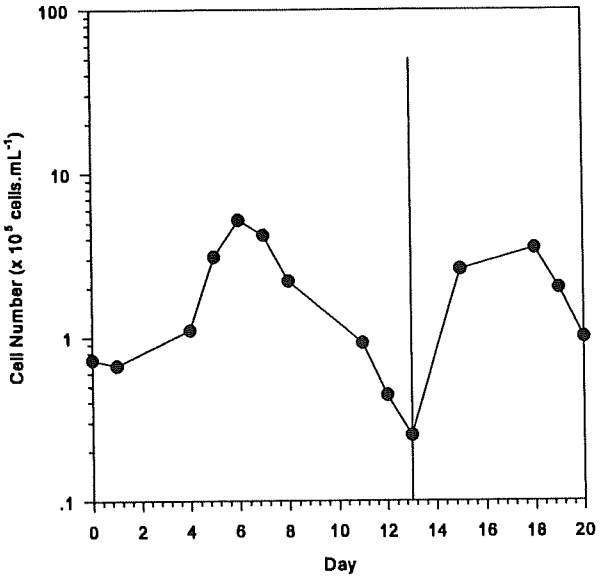

Figure 24 *Skeletonema costatum*. Growth in 38L BIOCOIL (BR1) in laboratory [Temp 23±1°C]

The experiment was repeated under the same conditions (**Figure 25**). After an 8 day lag period the culture grew rapidly reaching a cell density of about $2x10^6$ cells.mL^{-1} at which time 12L of the

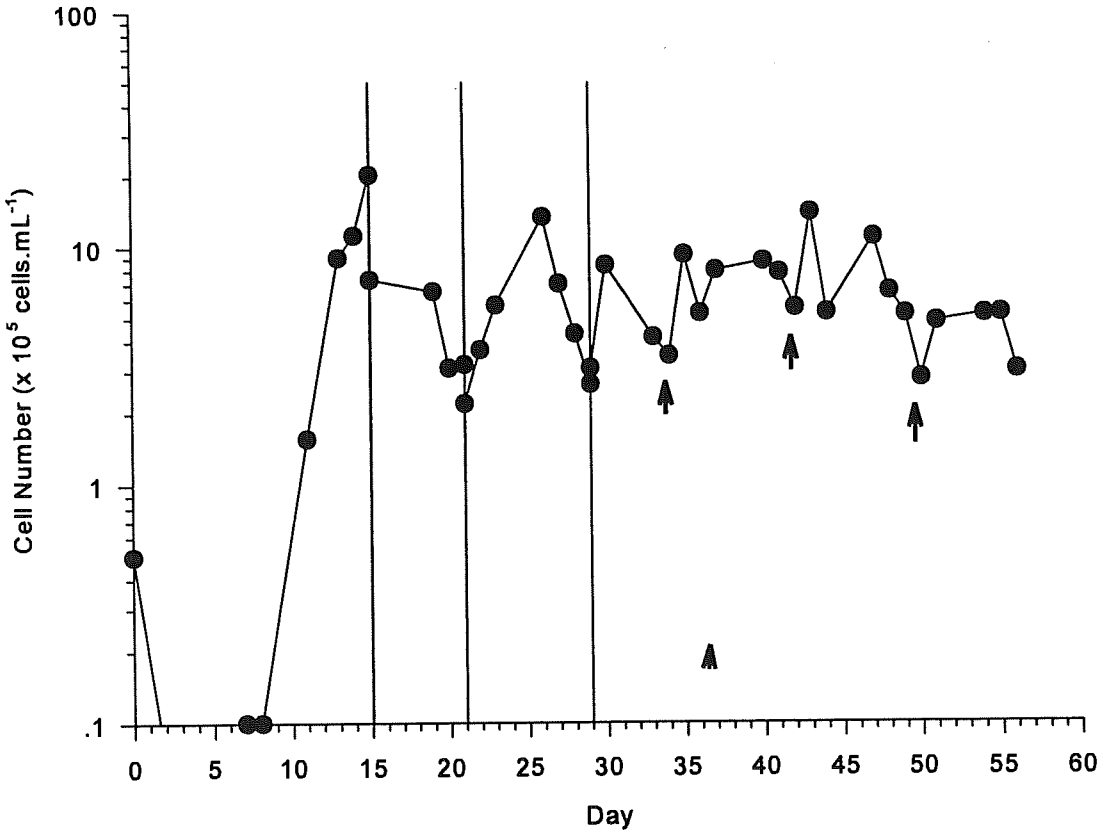

Figure 25 *Skeletonema costatum*. Growth in 38L BIOCOIL (BR1) in laboratory. Vertical lines = partial change of medium, Arrows = addition of extra Si.

culture was harvested and replaced with fresh medium. This was followed by a continuing decline in cell numbers until on day 21 another 12L of medium was harvested and replaced with fresh medium. Following this medium exchange the culture grew again for several days. When the culture had again begun to decline the harvest and medium replacement was repeated and growth resumed. The results so far suggested that the rapid decline in the culture was due to limitation by some critical nutrient and it was hypothesised that this nutrient was silicon. We therefore decided to add Si only when the culture declined rather than the partial medium replacement undertaken so far. The addition of Si (arrows in **Figure 25**) had an effect fairly similar to the 1/3 medium changes; i.e. the fall in cell numbers was arrested and growth resumed for a few days following the addition. This experiment was discontinued after 56 days.

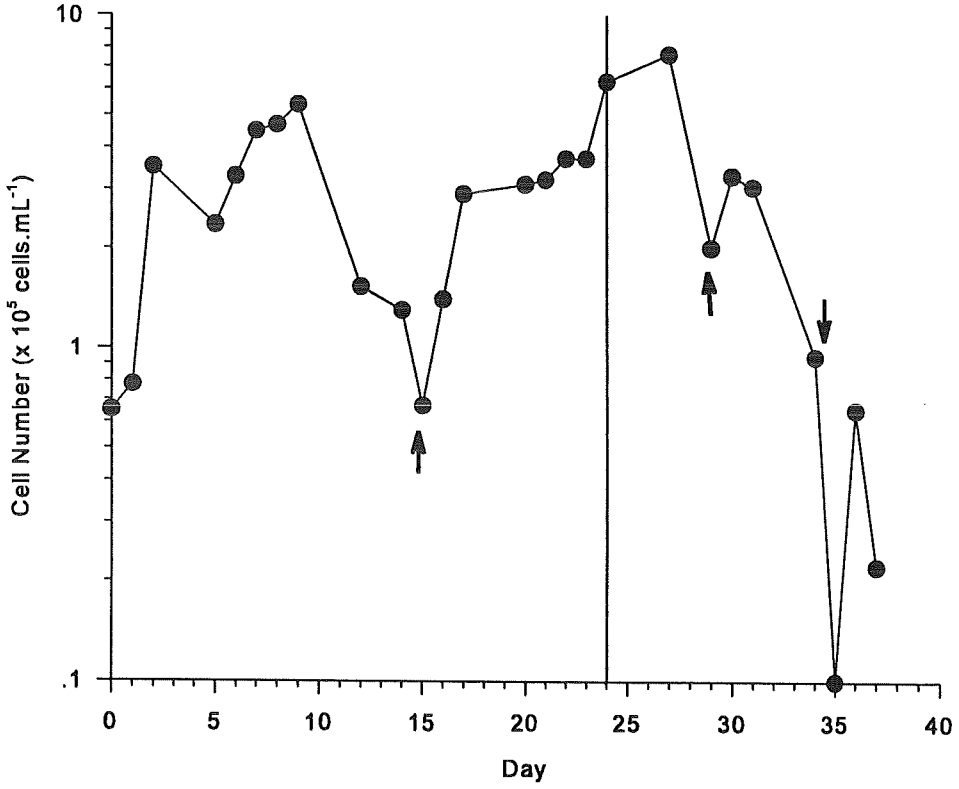

Figure 26 *Skeletonema costatum*. Growth in 34L BIOCOIL (BR1/6) in laboratory. Vertical line = partial change of medium; arrows = addition of Si. Temperature = 21±1°C.

The effect of Si addition was confirmed in the next experiment (**Figure 25, Figure 26**). Addition of Si only on day 15 arrested the decline in cell numbers and resulted in good growth. However, a partial medium change on day 24 resulted in only slight growth and subsequent addition of Si had little effect on the decline in cell numbers (**Figure 25, Figure 26**). The reasons for this are unclear. Unlike the other algae studied *Skeletonema* cultures sometimes failed to grow at all (not shown) and as the above experiments show, the response of *Skeletonema* to nutrient additions was also not wholly consistent.

In order to see how the culture grew in daylight an experiment in a 38L BIOCOIL in the glasshouse was carried out (**Figure 27**). After 4 days of growth the culture suddenly went into a major decline. This was probably due to difficulties in temperature control with the culture reaching >25°C between days 4 and 12. After day 12 the cooling system was boosted and the culture temperature could be maintained between 18 and 20.5°C. The cooler culture temperatures coincided with improved growth of the culture. As in the previous experiments in the laboratory,

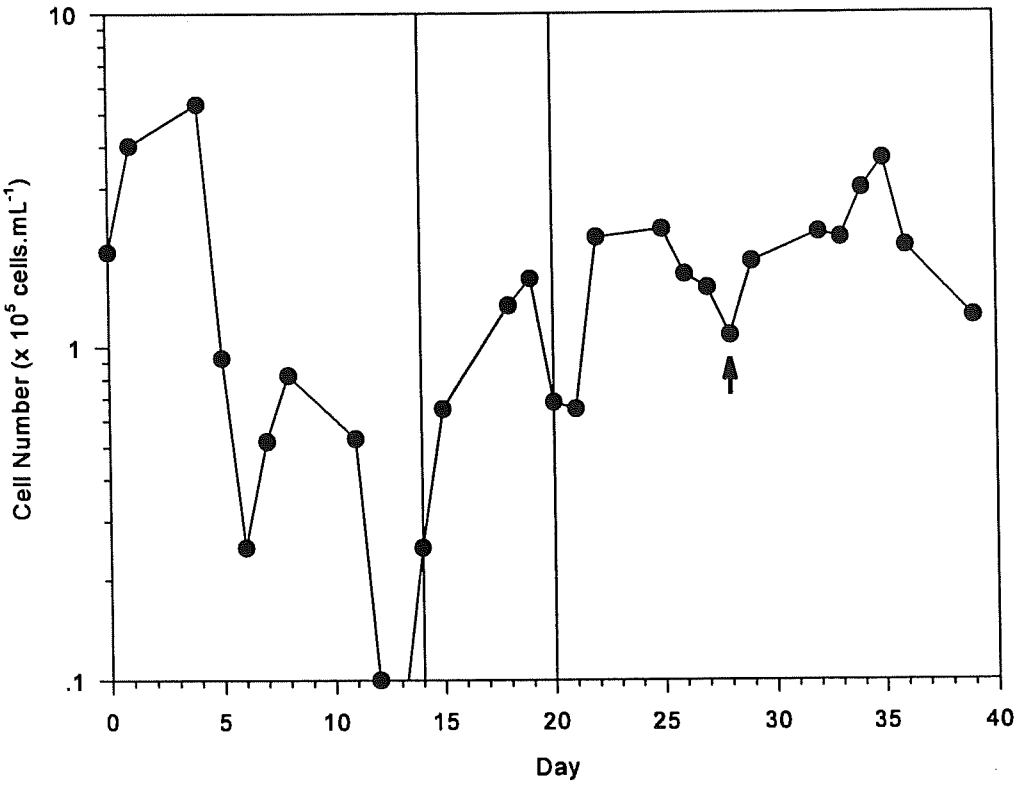

Figure 27 *Skeletonema costatum*. Growth in 38L BIOCOIL in glasshouse. Vertical lines = partial change of medium; Arrow = addition of Si only.

medium changes or the addition of Si arrested declines in cell numbers and resulted in growth. The maximum cell number achieved, however, were lower than those in the laboratory cultures. The reasons for this are not clear, but could possibly be due to the changed light environment.

The question of the maximum temperature and the effects of light were reexamined in another experiment carried out indoors (**Figure 28**). In this experiment the culture temperature was maintained at 24°C and media changes were undertaken approximately once weekly with Si additions between the medium changes.

The culture grew well at the higher temperature and reached a cell density of about 1 x 10^6 cells.mL^{-1}. Growth rate was fairly constant at about 1 doublings.day^{-1} over the 43 days of the experiment. After day 32 the maximum cell density achieved declined a little and the culture tended to adhere to the photostage tubes more.

The results of the last experiment showed that a culture of *Skeletonema* could be maintained in semi-continuous culture for at least 1.5 months in a BIOCOIL with careful management and that higher temperatures alone could not account for the poor growth in the glasshouse culture.

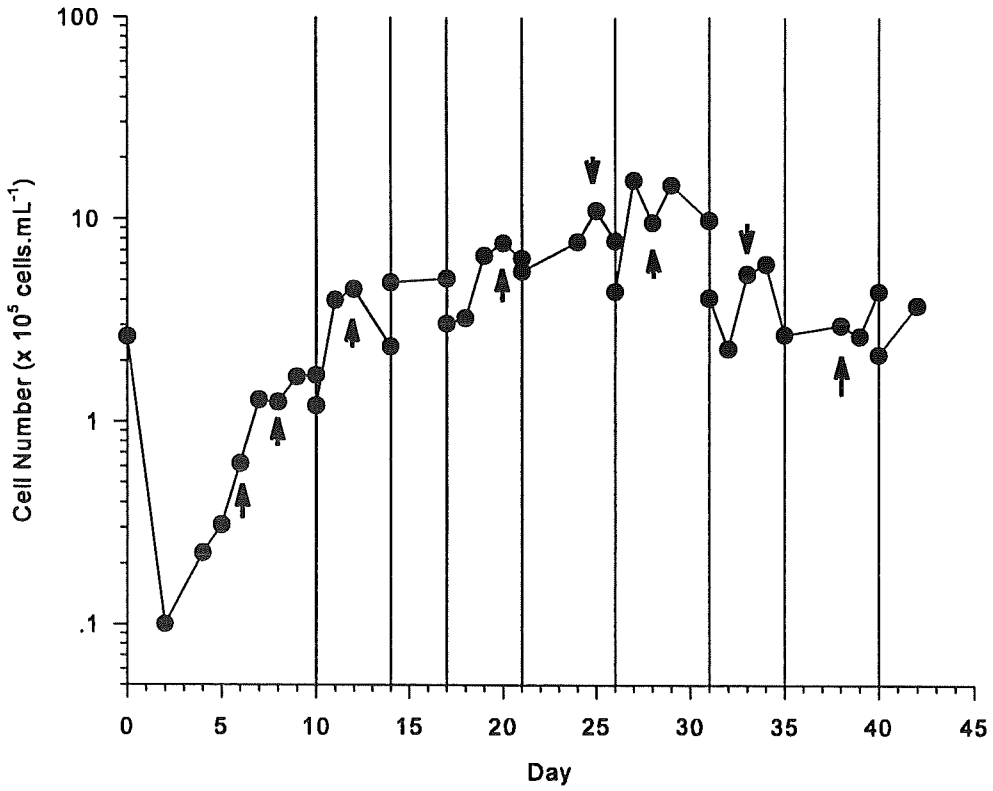

Figure 28 *Skeletonema costatum*. Growth in laboratory in 38L BIOCOIL (BR1). Vertical lines = partial medium change; arrows = addition of Si [Temperature = 24±0.5°C].

Chaetoceros gracilis and C. *calcitrans*

The experiments with these diatoms are still preliminary as much of the effort was focussed on *Skeletonema costatum* because of its importance to the prawn industry.

Figure 29 shows the growth of *Chaetoceros gracilis* in a 38L laboratory BIOCOIL. Initial growth was excellent and in order to see whether the culture was light limited we illuminated one side of the photostage with a quartz-halogen arc lamp which gave a mean irradiance of 2000 µmol

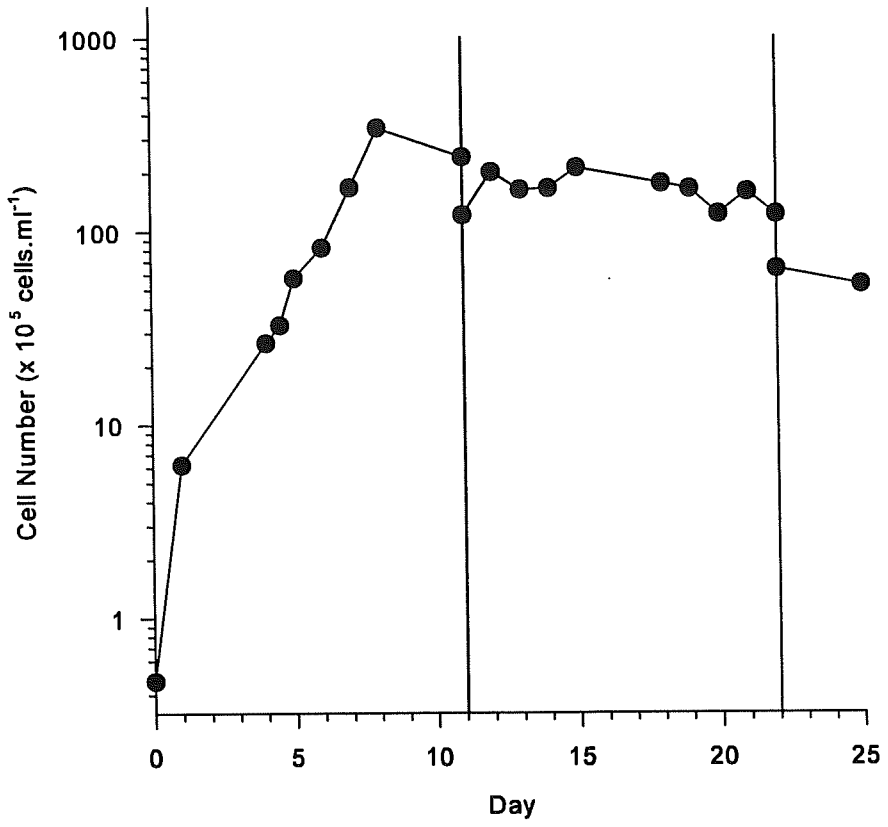

Figure 29 *Chaetoceros gracilis*. Growth in 38L BIOCOIL (BR1) in laboratory. After day 11 the culture was illuminated by a quartz halogen lamp on one side of the photostage. Vertical lines = partial change of medium.

photons.m^{-2}.sec^{-1} on the photostage surface. The additional heat load of this lamp gave some problems with the cooling system and on day 13 the culture reached a temperature of 27.3°C; despite this high temperature the culture survived. Once we boosted the capacity of the cooling system the temperature was maintained at about 22°C for the rest of the experiment however the cell numbers continued to decline gradually and the decline could not be arrested by a partial harvest and the addition of fresh medium. The reasons for this decline are not known.

The growth of *Chaetoceros calcitrans* was even less satisfactory (**Figure 30**). After about a week of rapid growth the culture declined rapidly. The results of other experiments results were equally poor. As this species was ranked of less importance to the aquaculture industry than others little

effort was made to determine the causes of the poor growth. Some preliminary observations however suggest that *Chaetoceros* is more shear sensitive than any other algae so far studied.

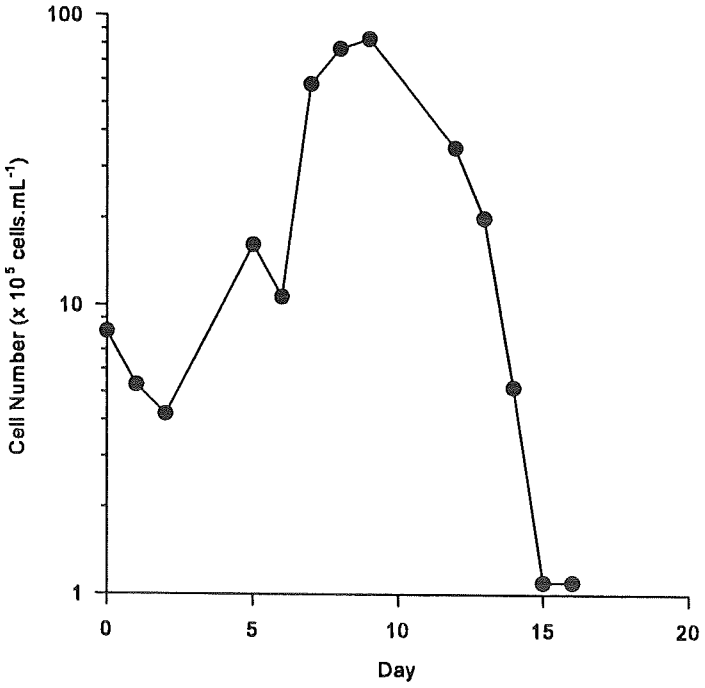

Figure 30 *Chaetoceros calcitrans.* Growth on 38L BIOCOIL in laboratory.

Chroomonas salina

Chroomonas salina grew well in the laboratory in a 38L BIOCOIL (**Figure 31**). Regular 20L changes in the medium maintained a constant growth rate and cell yield for about 40 days. If the culture medium was not changed at about 7 day intervals the culture began to decline (Cf. **Figure 31** days 49-70) but recovered again when the medium was partially changed. By about day 96 the culture had become contaminated with a flagellate and the experiment was terminated soon thereafter.

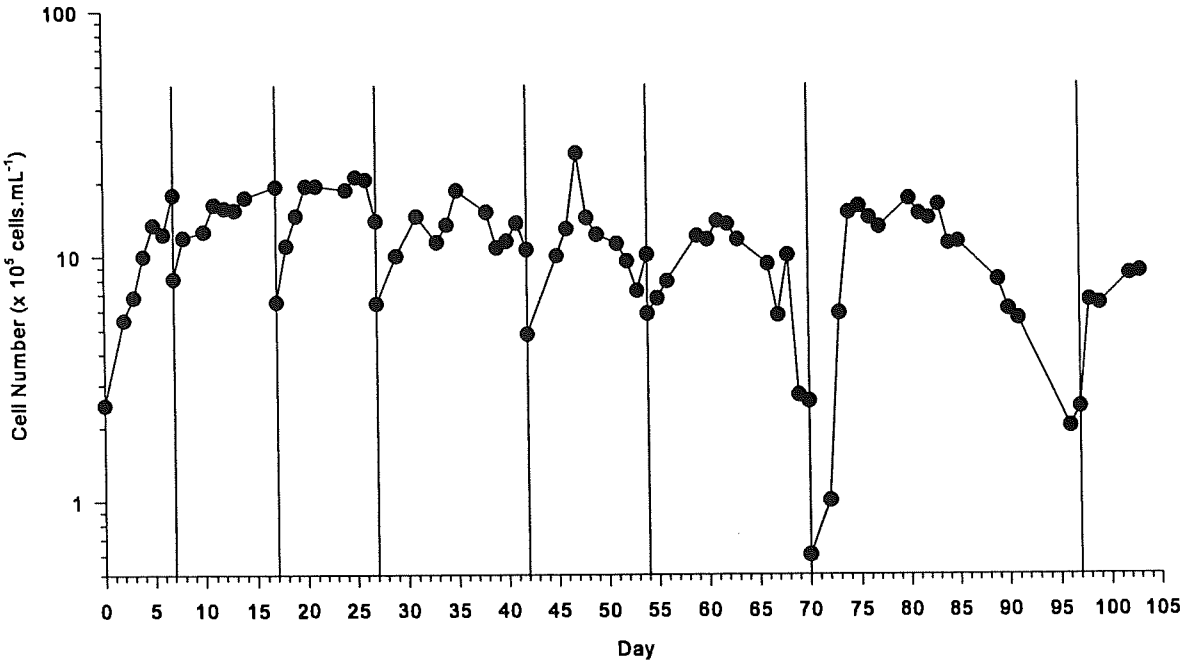

Figure 31 *Chroomonas salina*. Growth in the laboratory in a 38L BIOCOIL (BR3) [Temperature 22±1°C] T3-34.

Large-scale culture

The experiences with the laboratory-scale cultures and the design and operation of the small laboratory BIOCOILs were used to design the 550L pilot-scale BIOCOILs (see page 14 and Borowitzka, 1996 for discussion of design criteria). The outdoor pilot-scale photobioreactors were finally operational[2] in July 1995.

Figure 32 shows the results of the first full-scale culture experiment in these reactors using *Tetraselmis chuii*. The top panel in this figure also shows the weather conditions. This alga was selected for the first run as it had been proven to be very resilient in the laboratory-scale trials. The culture of *Tetraselmis chuii* in the pilot-scale reactor grew well despite some significant equipment failures, especially failures of the compressor which provides the air supply to the reactor (see **Figure 32**). Failures of the circulation system for about 24h or less had only a temporary effect on the culture, whereas longer failures or regular intermittent failures reduced the maximum cell number considerably. However, the algal system was quite resilient as is shown by the relatively rapid recovery of the culture once the air supply, and thus the circulation, was restored. This pilot reactor was operated for a period of 160 days when the experiment was terminated for modifications to the reactor and for trials with other species.

It is useful to compare these results with **Figure 20** which shows the results of a parallel experiment in the glasshouse in a 60L reactor. The growth rate in the pilot-scale reactor and the small reactor in the glasshouse was the same indicating that the smaller 'laboratory-scale' BIOCOILs in the glasshouse under the same light regime are excellent models for the pilot-scale units.

The second species trialed in the pilot-scale reactor was *Isochrysis* (**Figure 33**). Initially this culture did not grow well and, due to problems with air supply to the airlift, many of the cells tended to settle out of the culture; this settling out probably accounts for a large part of the declining cell numbers. The generally overcast or rainy weather at this time may also have contributed to the settling. On day 25 additional nutrients were added and the air-flow to the airlift adjusted following which good growth was observed. The culture grew well despite some compressor failures, however after the partial medium change on day 42 the culture had become contaminated with a rotifer and the experiment was terminated on day 55.

These first two experiments in the 550L pilot-scale BIOCOILs highlighted several problems with their operation, design and ancillary equipment. The controllers on the compressors were found to be inadequate for the workload and maintaining the culture unialgal and free of contaminants was also a problem. Furthermore, flow rates were also not as high as required. The compressor problems were solved by installing a second backup compressor as well as more robust controllers. The flow-rate was improved by altering the relative levels between the photostage and the airlift, and in order to reduce the possibility of contamination the air filter for the airlift was changed and the design of the air outlet modified before the next series of experiments.

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[2]*Construction of the pilot-scale reactors was delayed by several factors, the main one being the flooding on the Nullarbor Plain which delayed delivery of the plastic tubing for the reactors by about 1.5 months, which, in turn led to a further delay in construction as the engineering company now had several jobs to finish at the same time. Some initial construction problems were also encountered in the manufacture of the airlifts.*

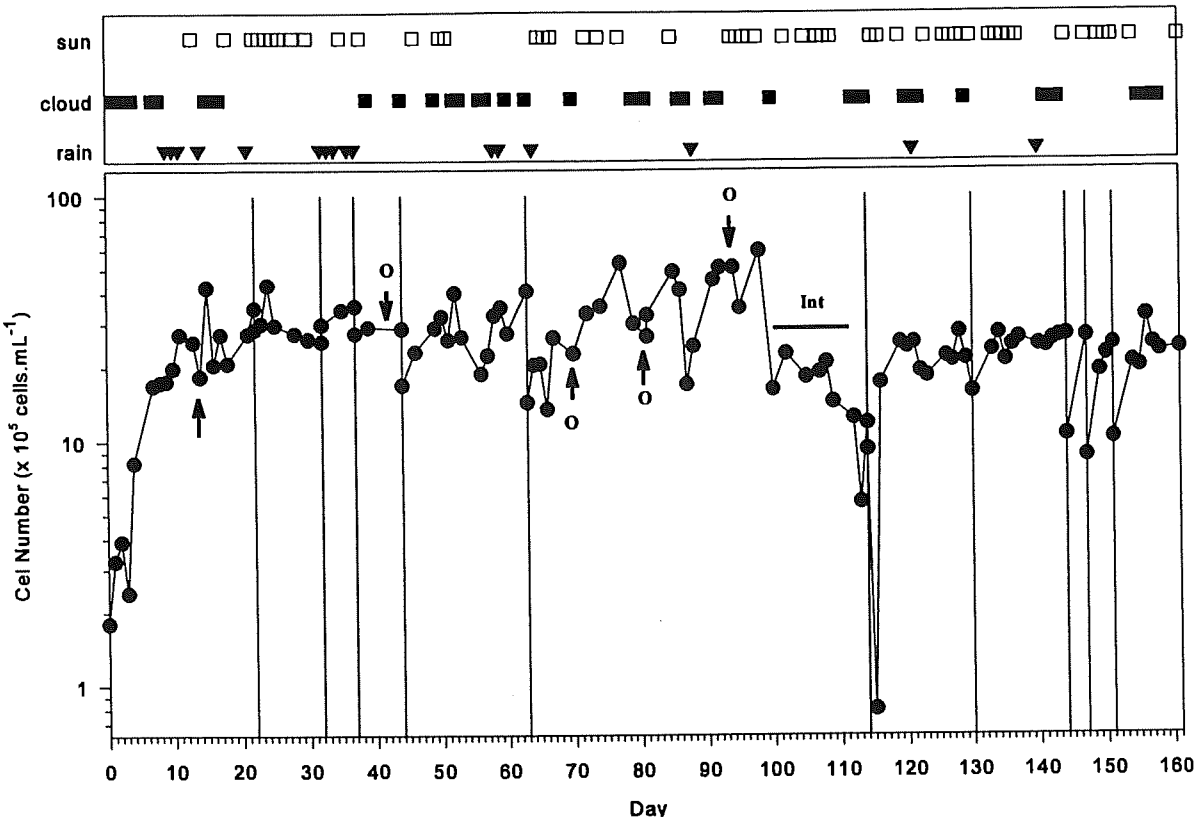

Figure 32 *Tetraselmis chuii.*

Bottom panel: Growth in pilot-scale BIOCOIL outdoors. the culture was initially operated with only part of the photostage tubing and on day 14 (up arrow) all the 'wraps' of tubing were connected. Vertical lines = partial replacement of culture with fresh medium; **O** = compressor failure (no circulation of culture for up to 24h); **Int** = intermittent compressor operation (i.e. intermittent culture circulation) due to compressor problems.

Top panel: Weather conditions during this experiment.

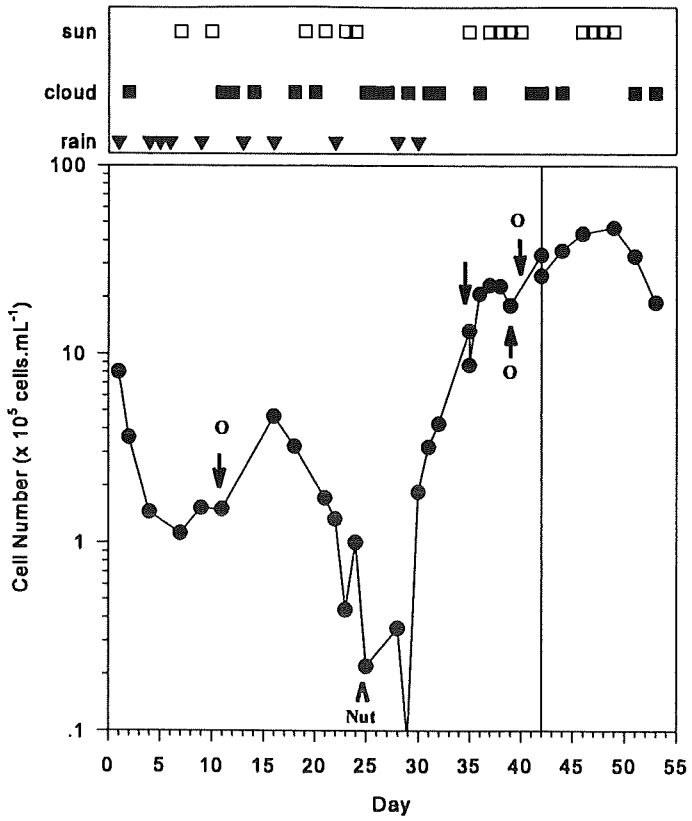

Figure 33 *Isochrysis galbana* (T-iso).
Bottom panel: Growth in pilot-scale BIOCOIL outdoors. The culture was initially operated with only one third of the photostage tubes and on day 34 (down arrow) another third of the 'wraps' was added to the circuit. On day 42 the final 'wraps' were added. **O** = compressor failure (no circulation) for up to 24h; **Nut** = addition of nutrients; vertical line = partial harvesting and replacement with fresh medium.
Top panel: weather conditions during this experiment.

Several growth trials in the pilot-scale BIOCOILs after these initial experiments highlighted further problems with contamination and with the chemical sterilisation process. In particular, *Tetraselmis* proved to be difficult to kill by the chlorination routine used for the laboratory reactors. This alga occurred as a contaminant in several runs when it had been grown previously in that particular BIOCOIL. Several trials were therefore undertaken to develop a more reliable chlorination protocol. Furthermore, additional modifications to the reactors were made to improve temperature control, install a CO_2 supply system and to improve the way the photostage tubes were mounted on their metal frame so that flow was more even and to cope with the significant expansion of the tubes during the day.

Figure 34 shows the results of another long-term run with *Isochrysis* in a pilot-scale BIOCOIL following some of these modifications and improvements. The alga grew very well and after 1 month of normal operation the CO_2 system was added in a pH-stat basis to determine whether this would enhance cell yield. The inclusion of the CO_2 supply did not improve the cell yield, rather the maximum cell number achieved declined. There are two possible causes for this

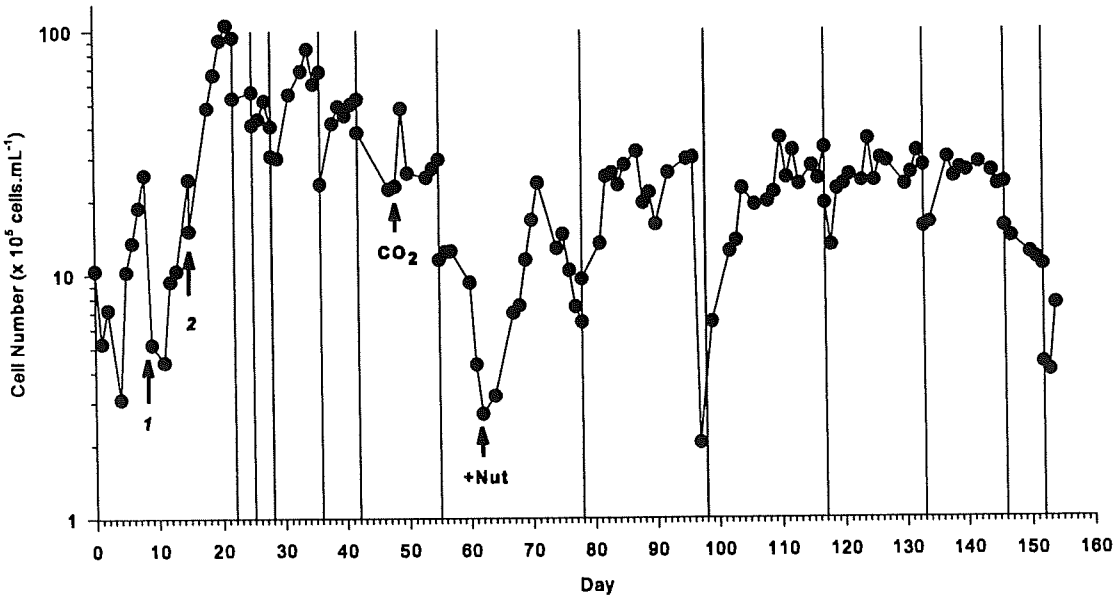

Figure 34 *Isochrysis galbana* (I-iso). Growth in pilot-scale BIOCOIL outdoors. Vertical lines indicate partial harvest and replacement with fresh medium. Until day 9 the reactor was operated with only 1/3 of the 'wraps' of the photostage. *1* indicates addition of another 1/3 of the 'wraps' and *2* indicates when all the 'wraps' of the photostage were connected. The CO_2 system was switched on on day 48 (arrow) and *+Nut* indicates addition of extra nutrients following a decline in cell numbers.

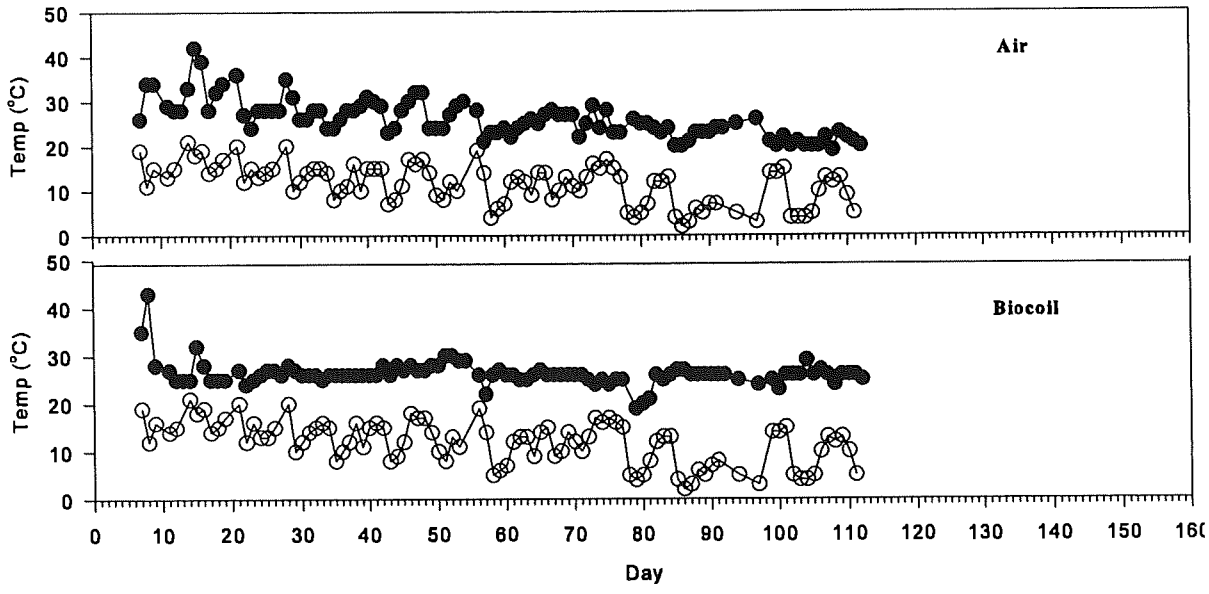

Figure 35 Maximum (●) and minimum (○) air and BIOCOIL temperatures for the culture shown above.

in maximum cell number: (1) due to some unexplained action of the CO_2, (2) reduced light reaching the algae as a result of clouding of the PVC tubes under the influence of high UV outdoors, or (3) lower night-time temperatures. The latter explanation seems more likely, based on the results of laboratory experiments and the observation that over the first 110 days there was a gradual decline in the minimum night air and BIOCOIL temperature (**Figure 35**). The drop in cell numbers between days 55 and 62 and also the drop around day 90 coincide with particularly cold nights resulting in BIOCOIL temperatures of less than 6°C (**Figure 35**). The culture, however, recovered very well from these declines and this was clearly helped by additional nutrients. The lower maximum cell density of approximately 1.2 x 10^6 cells.mL^{-1} (= approximately 1.1g dry weight.L^{-1}) is however still very satisfactory. Growth rates remained high and the culture was stable for about 150 days.

Pavlova lutheri also grew well in the 550L BIOCOIL (**Figure 36**). During the first week there was no growth and this appears to have been due to a too slow flow rate. After increasing the flow rate on day 9 the cell numbers increased rapidly and on day 21 all 15 'wraps' were connected to the system. The culture was stable for the 64 days of the experiment at a cell density of about 1.3 x 10^6 cells.mL^{-1}.

Figure 37 shows the culture of *Chroomonas salina* in the 550L BIOCOIL. This alga also grew well, reaching cell densities of about 1.15 x 10^6 cells.mL^{-1}. However, after the partial media change on day 21, the culture became heavily contaminated with a green flagellate (cell densities of up to 1.1 x 10^6 cells.mL^{-1}) and a rotifer (up to 1.5 x 10 cells.mL^{-1}). A likely cause for this contamination was a crack which had developed in the airlift header tank.

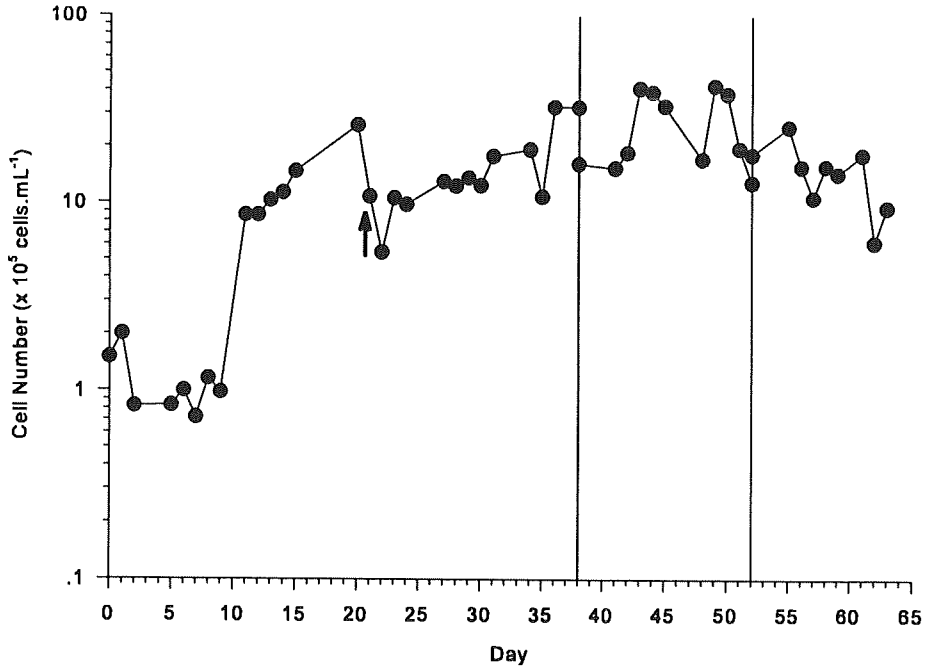

Figure 36 *Pavlova lutheri.* Growth in pilot-scale BIOCOIL outdoors. Arrow = connection of all 'wraps' to the culture system. vertical lines = approximately 50% harvest and replacement with fresh medium.

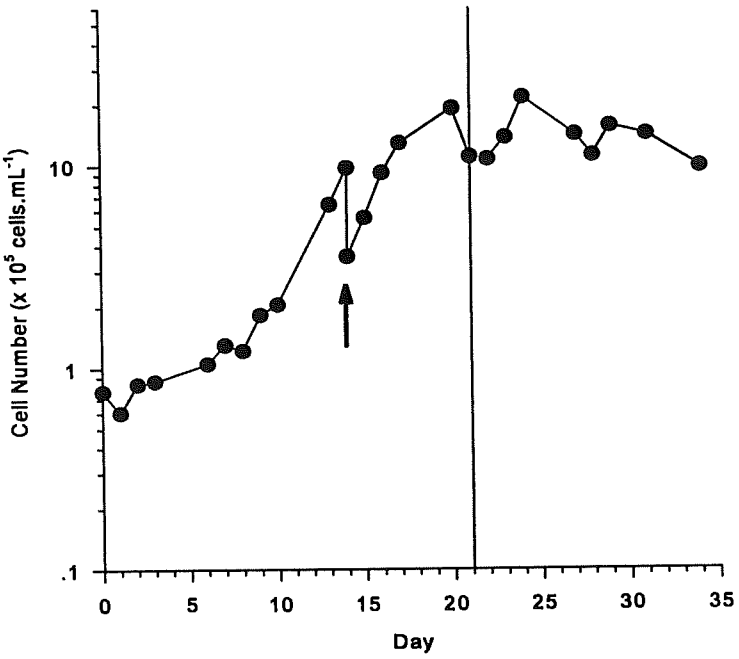

Figure 37 *Chroomonas salina*. Growth in pilot-scale BIOCOIL outdoors. Arrow = inclusion of all 'wraps' in the culture system. vertical line = partial harvest and replacement with fresh medium.
Following this addition the culture became heavily contaminated with a green flagellate and a rotifer.

Temperature control in pilot-scale BIOCOILs

An important requirement for the outdoor BIOCOILs is the ability to control temperature. For economical reasons a heat exchanger similar to that used in the laboratory BIOCOILs is not feasible. The 550L outdoor BIOCOILs were therefore fitted with an evaporative cooling system in which a thin film of water was pumped over the surface of the photostage coils when the culture temperature exceeded a particular set point. The culture temperature was measured with a temperature probe mounted on the surface of the tubes and insulated from the air. This probe was connected to a temperature controller. An air temperature reference probe was also included.

The following figures (**Figure 38** to **Figure 40**) show some of the temperature data. In the mornings the coil temperature rose much more rapidly than the air temperature as the photostage coils act similar to a solar hot water system. Once the maximum set temperature was reached the water cooling system cut in and cooled the reactor so that the temperature of the culture oscillated several degrees around the set point. The timing and scale of these oscillations is a function of the thermal mass of the total culture (photostage plus manifolds plus airlift). After sunset the coils cooled generally slower than the air temperature. The rate of cooling is dependent upon both the air temperature and on the wind chill factor (i.e. cooling is slower on calm nights). The morning heating of the coils was largely unaffected by weather conditions (eg. **Figure 40**) and the coils operated at their set maximum temperature irrespective of air temperature (eg. **Figure 39**).

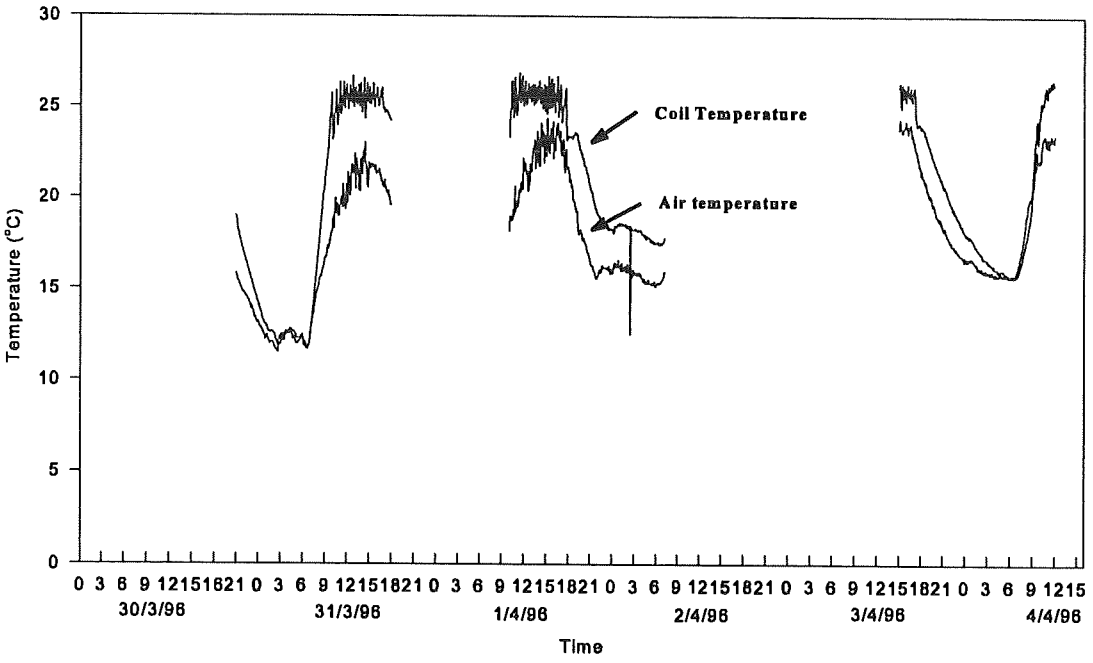

Figure 38 Air and culture temperature in 550L outdoor BIOCOIL.

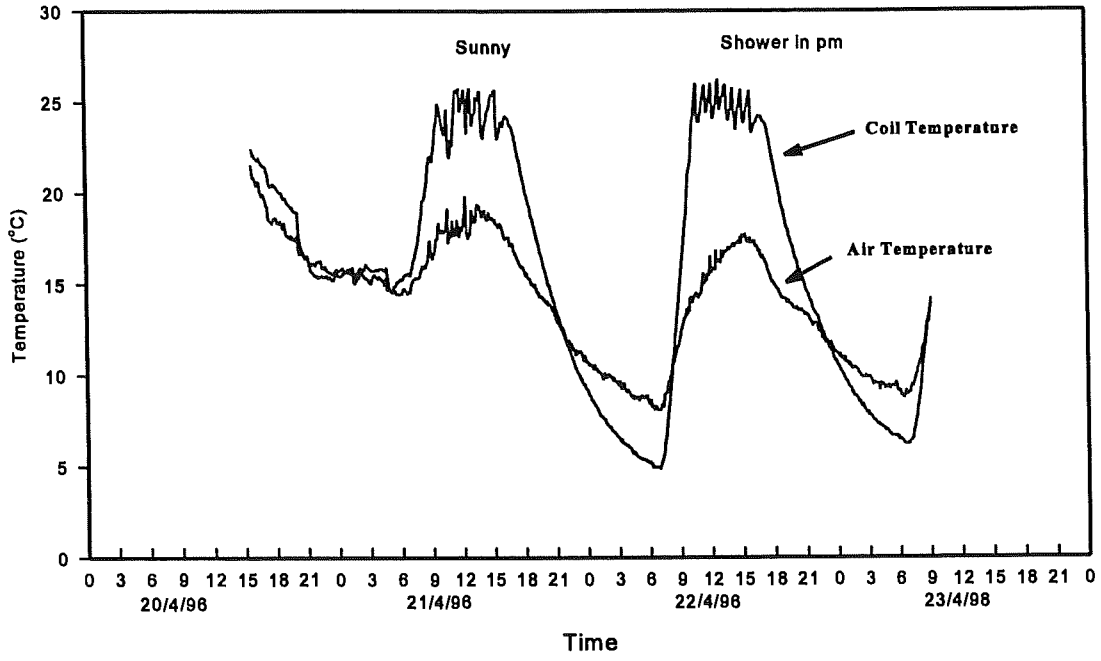

Figure 39 Air and culture temperature in 550L outdoor BIOCOIL showing effect of weather and cool night temperatures.

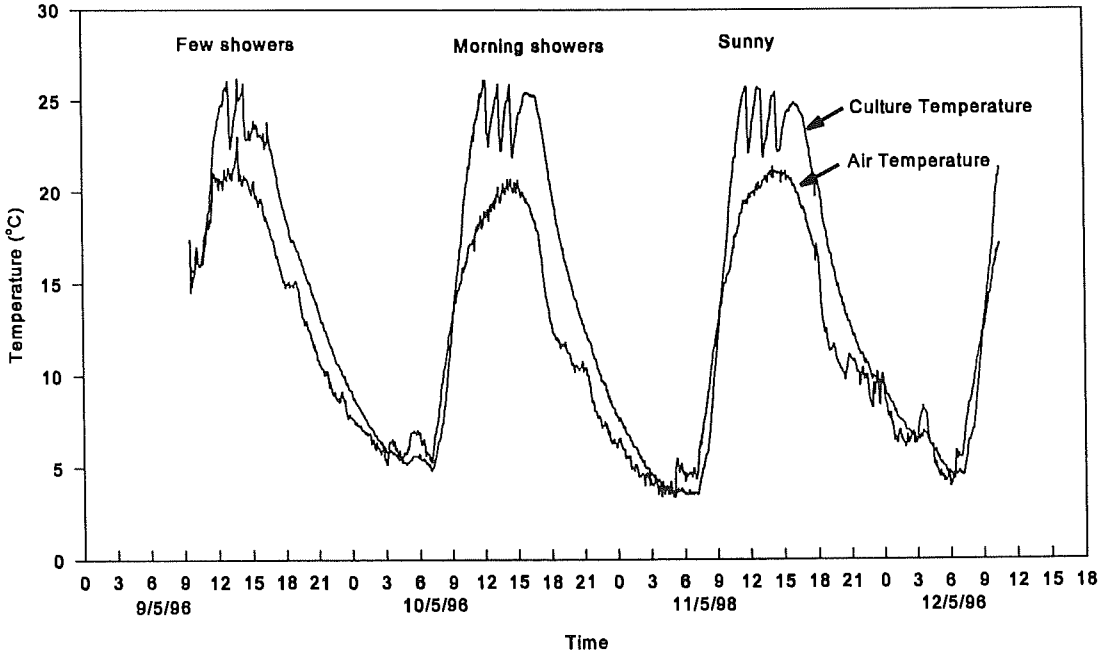

Figure 40 Air and culture temperature in 550L outdoor BIOCOIL showing effects of weather.

Figure 41 shows a detailed comparison of the relationship between the BIOCOIL temperature and ambient climatic conditions. It shows that the culture temperature was little influenced by air temperature and relative humidity. The small changes in the maximum culture temperature were mainly due to variations in wind.

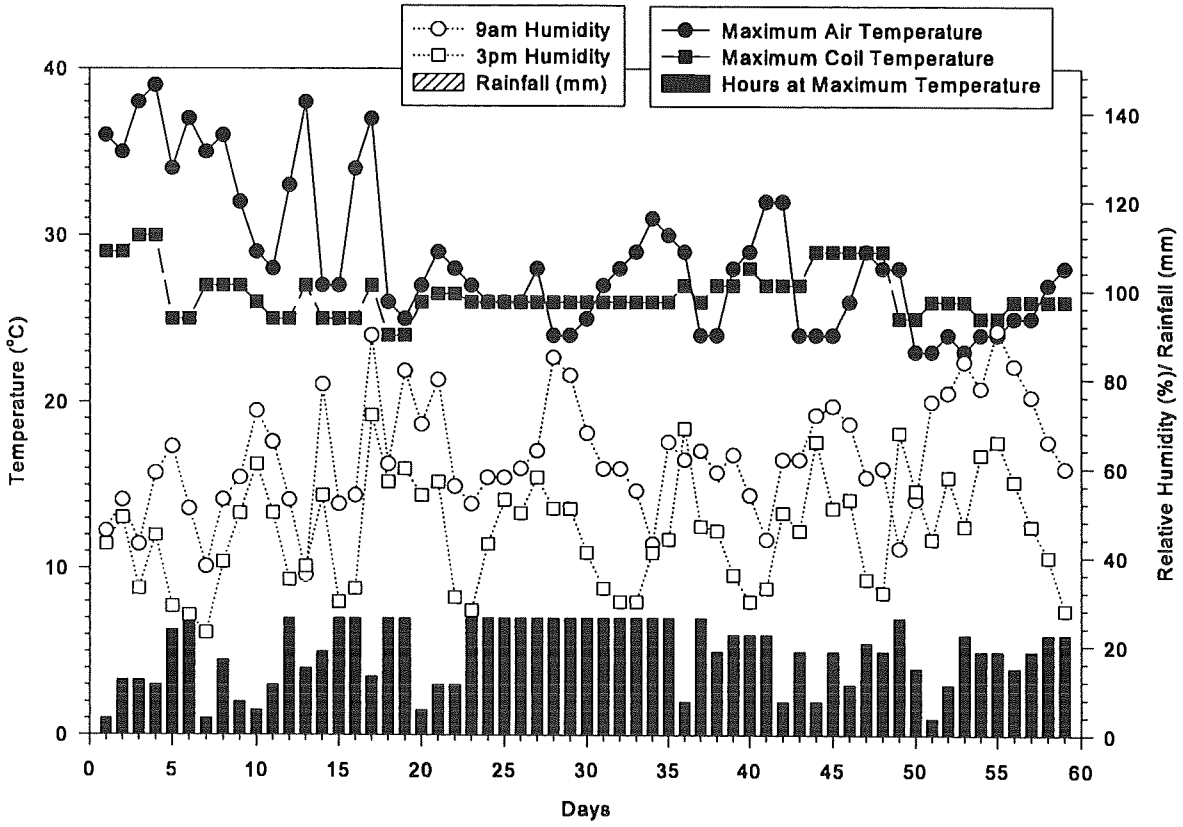

Figure 41 Relationship between climatic conditions (relative humidity, air temperature, rainfall) and coil temperature (maximum temperature and number of hours at maximum temperature per day) for 550L BIOCOIL. Day 0 = 22 February 1996. Climatic data form Perth Meterological Office. The higher BIOCOIL temperatures in the first 4 days were due to uneven flow of the cooling water over the photostage and this was rectified thereafter.

Limits to growth

An important question in optimising the design and operation of the BIOCOIL is an understanding of the limits to growth. In large-scale commercial algal cultures light is the main limiting factor (Richmond *et al.*, 1990) and pond cultures are grown at as shallow a depth as is possible. In paddlewheel ponds the minimum pond depth is largely determined by the hydraulics of the system and needs to be sufficient for adequate circulation of the culture by paddlewheels (Oswald, 1988). This depth is about 30 cm. Similarly the large open ponds used to culture *Dunaliella salina* in Australia are operated at about 30-40 cm. The long light path in these ponds means that the maximum biomass achievable is generally less than 1g dry wt.L^{-1}. Growth rates can be improved by managing the algal density (i.e. less dense cultures mean less self-shading of the cells and thus better growth; Richmond *et al.*, 1980; Vonshak *et al.*, 1982) however this approach is not feasible in the very large-scale commercial systems.

One of the advantages of a tubular photobioreactor such as the BIOCOIL is the short light path (about 25 mm) and the ability to work outdoors in natural daylight. The BIOCOIL also allows a greater degree of temperature control and, as the system is closed, prevents contamination by other organisms. One of the disadvantages of tubular photobioreactors is that as the culture flows through the tubes and as the algae photosynthesise the O_2 concentration in the water increases. High concentrations of O_2 may lead to photorespiration (the light stimulated oxidation of the products of photosynthesis to CO_2) thus inhibiting photosynthetic O_2 evolution (Beardall, 1989; Richmond *et al.*, 1993). It is therefore essential to understand the sensitivity of the species cultured to oxygen tension at various temperatures so that the temperature can be optimised.

Effect of oxygen

In order to evaluate the importance of photorespiration in the culture of the algae experiments were carried out to determine the sensitivity of the algae to O_2.

***I. galbana*:** The gross photosynthetic rate of *I. galbana* decreased with increasing O_2 concentration above 50% sat_{air} for all conditions except for the 23 °C treatment at 1200 µmol photons.m^{-2}.sec^{-1} which was inhibited at concentrations above 80% sat_{air}. The rate of decrease in gross photosynthetic rate (O_2 inhibition rate) was affected by both irradiance and temperature (**Figure 43**a). At 2500 µmol photons.m^{-2}.sec^{-1} much higher inhibition was observed at 26°C than at the lower temperatures (**Figure 43**b, Table 6).

The gross photosynthetic rate at 1200 µmol photons.m^{-2}.sec^{-1} was highest at 23 C with a temperature specific maximum of 177.2 µmoles O_2. g Chl a^{-1}.min^{-1}. The gross photosynthetic rate declined with decreasing temperature. At 2500 µmol photons.m^{-2}.sec^{-1} the highest temperature specific maximum gross photosynthetic rate was observed at 26°C with a value of 305.75 µmoles O_2.g Chl a^{-1}.min^{-1}. This value was approximately 1.7 times higher than the highest TSM gross photosynthetic rate at 1200 µmol photons.m^{-2}.sec^{-1}. The temperature specific maximum gross photosynthetic rate was very similar for both the 20°C and 23°C treatments (**Figure 43**).

***T. chuii*:** The O_2 inhibition rate of *T. chuii* also was affected by both temperature and irradiance (**Figure 45**). Increasing temperature lead to an increase in both the gross photosynthetic rate and the O_2 inhibition rate at both 1200 and 2500 µmol photons.m^{-2}.sec^{-1}. Increasing irradiance

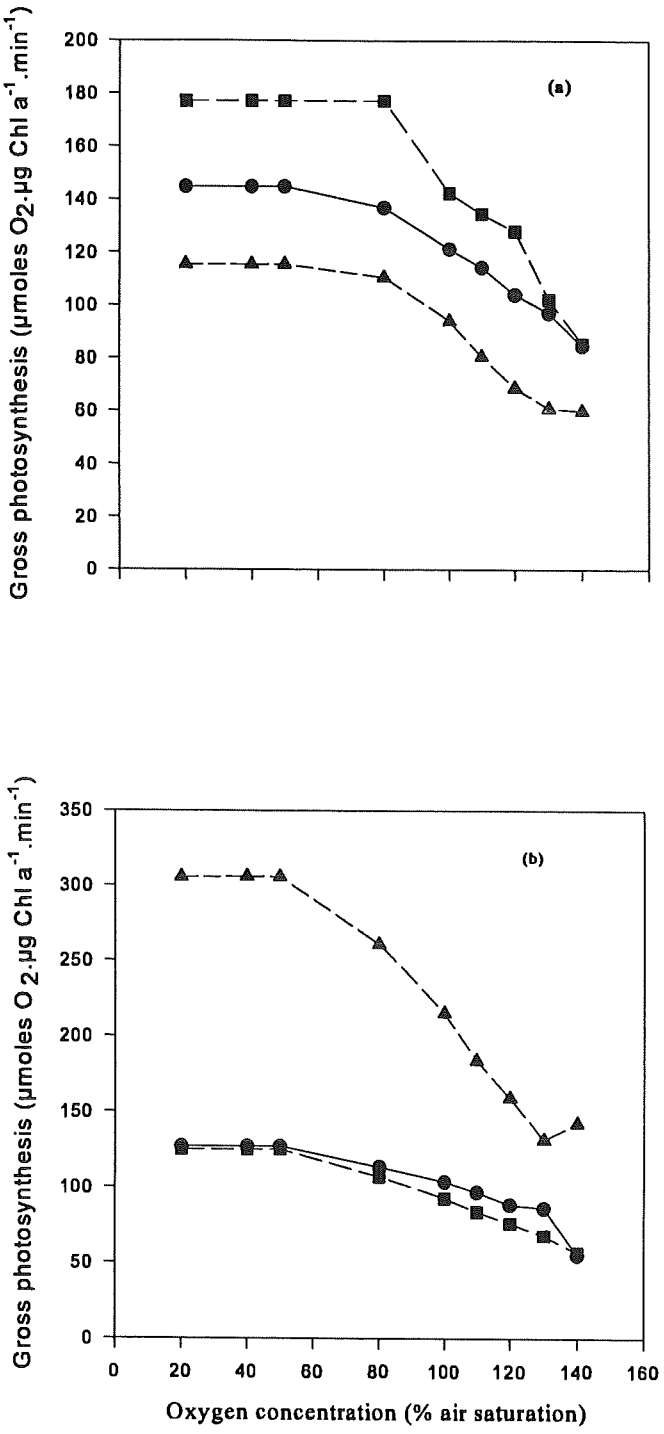

Figure 43 The effect of oxygen concentration on the gross photosynthetic rate of *Isochrysis galbana* at (a) 1200 µmol photons.m^{-2}.sec^{-1}, (b) 2500 µmol photons.m^{-2}.sec^{-1} and three temperatures. (●) 20°C (36.3 µg Chl *a*); (■) 23°C (43.7 µg Chl *a*), (▲) 26°C (25.5 µg Chl *a*).

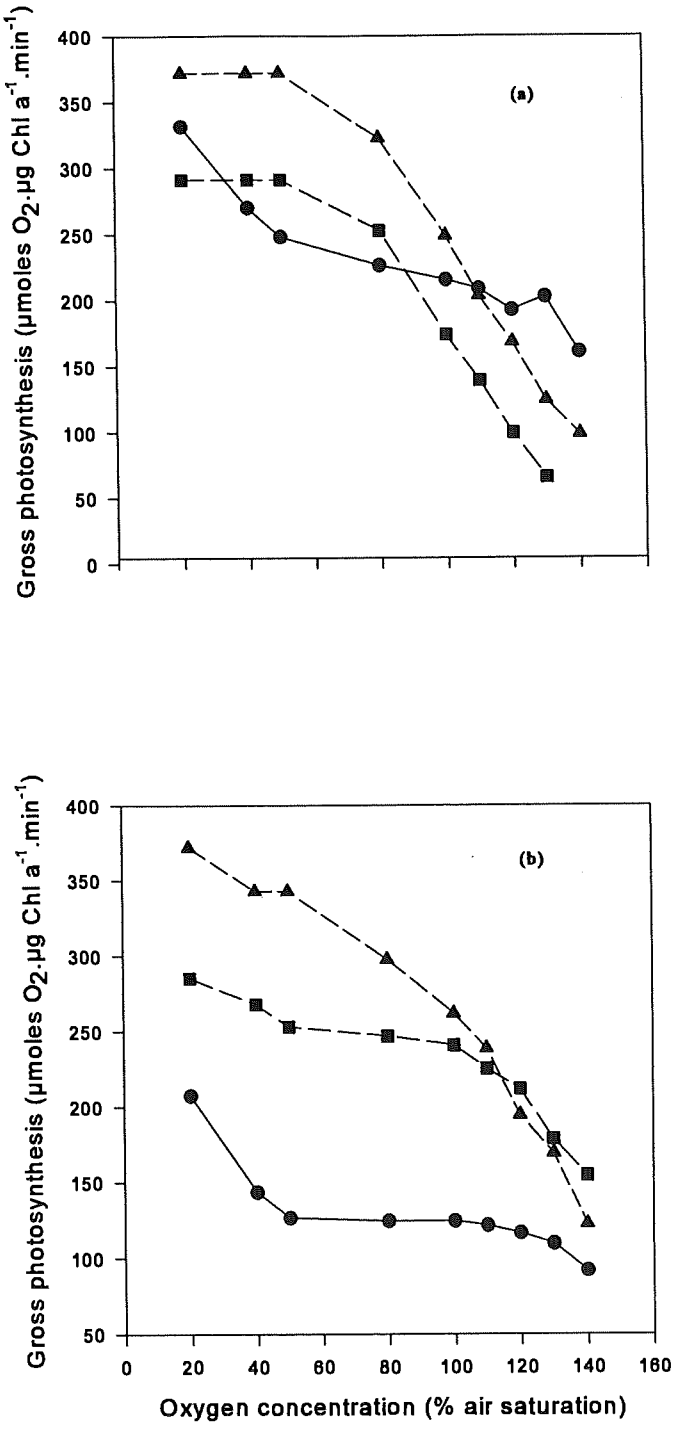

Figure 45 The effect of oxygen concentration on the gross photosynthetic rate of *Tetraselmis chuii* at (a) 1200 µmol photons.m^{-2}.sec^{-1}, (b) 2500 µmol photons.m^{-2}.sec^{-1} and three temperatures. (●) 20°C (29.5 µg Chl *a*); (■) 23°C (30.5 µg Chl *a*) (▲) 26°C (36.3 µg Chl *a*).

decreased the O_2 inhibition rate as well as decreasing gross photosynthetic rate at all three temperatures (**Figure 45**). Increasing irradiance also shifted the point at which O_2 inhibition began from 50% sat_{air} for the 23°C and 26°C treatments at 1200 µmol photons.m^{-2}.sec^{-1} to 20% sat_{air} for the same treatments at 2500 µmol photons. m^{-2}. sec^{-1}. O_2 inhibited the gross photosynthetic rate at greater than 20% sat_{air} at 20°C at both irradiances tested (**Figure 45**).

The rate of inhibition at 2500 µmol photons.m^{-2}.sec^{-1} reached a plateau between 50% sat_{air} and 110% sat_{air} for both the 20°C and 23°C treatments, after which it began to increase again until 140% sat_{air} to the original rate before 20% sat_{air}. The O_2 inhibition rate at 20°C at 1200 µmol photons.m^{-2}.sec^{-1} showed a similar trend (Table 6).

***T. suecica*:** O_2 inhibition was observed for *T. suecica* at greater than 50% sat_{air} at all temperatures and irradiances except at 20°C and 1200 µmol photons.m^{-2}.sec^{-1} at which inhibition was observed only at greater than 80% sat_{air} (**Figure 47**). Increasing temperature at 1200 µmol photons.m^{-2}.sec^{-1} led to an increase in the gross photosynthetic rate at all oxygen concentrations. It also increased the O_2 inhibition rate (Table 6). At 2500 µmol photons.m^{-2}.sec^{-1} the O_2 inhibition rate decreased with increasing temperature (Table 6). The O_2 inhibition rate was less at 2500 µmol photons.m^{-2}.sec^{-1} for all temperatures except 20°C (**Figure 47**).

Increasing irradiance decreased gross photosynthetic rate at all O_2 concentrations except at 20°C where it increased (**Figure 47**).

***S. costatum*:** O_2 inhibition was observed for *S. costatum* at greater than 50%$_{air}$sat for all treatments except at 26°C, 1200 µmol photons.m^{-2}.sec^{-1} and 20°C, 2500 µmol photons.m^{-2}.sec^{-1} for which inhibition occurred at greater than 80% sat_{air} (**Figure 49**).

Increasing temperature at 1200 µmol photons.m^{-2}.sec^{-1} lead to an increase in the O_2 inhibition rate (Table 6). Increasing temperature at this irradiance also resulted in an decrease of the temperature specific maximum gross photosynthetic rate over all oxygen concentrations (**Figure 49**).

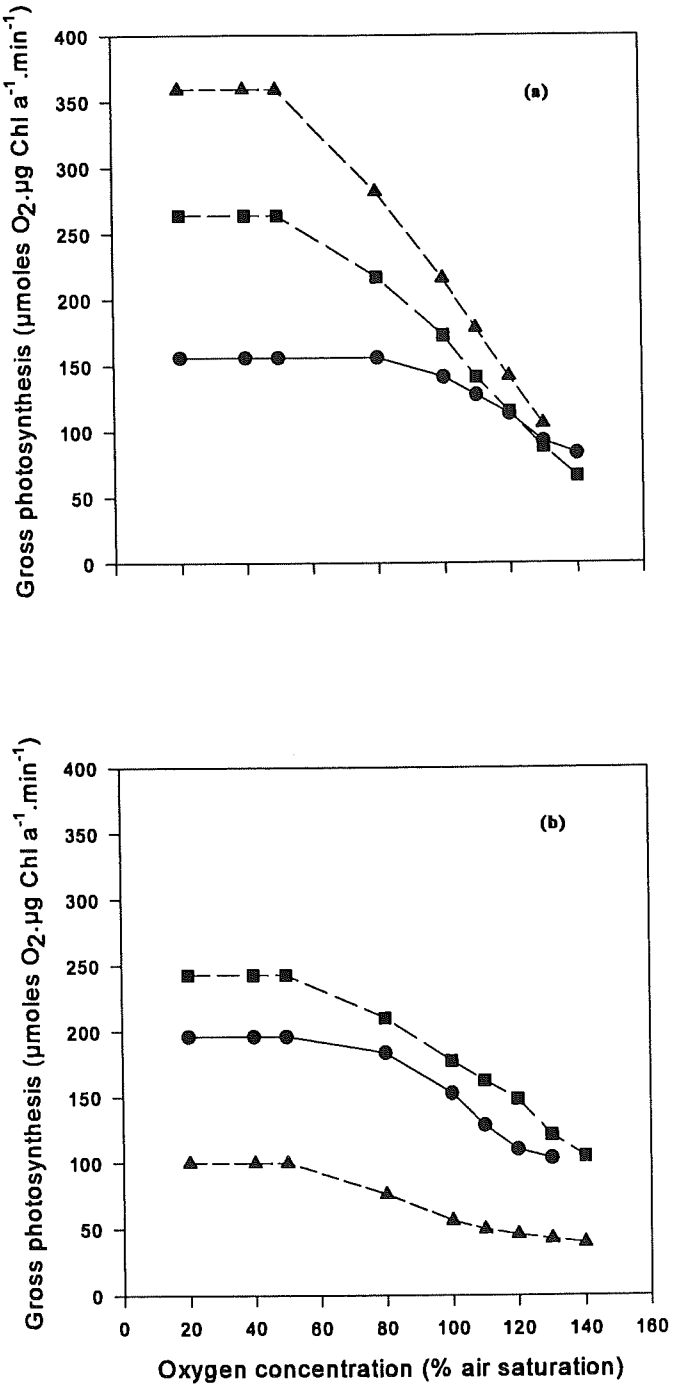

Figure 47 The effect of oxygen concentration on the gross photosynthetic rate of *Tetraselmis suecica* at (a) 1200 µmol photons.m^{-2}.sec^{-1}, (b) 2500 µmol photons.m^{-2}.sec^{-1} and three temperatures. (●) 20°C (33.2 µg Chl *a*); (■) 23° C (24.4 µg Chl *a*), (▲) 26°C (29.3 µg Chl *a*).

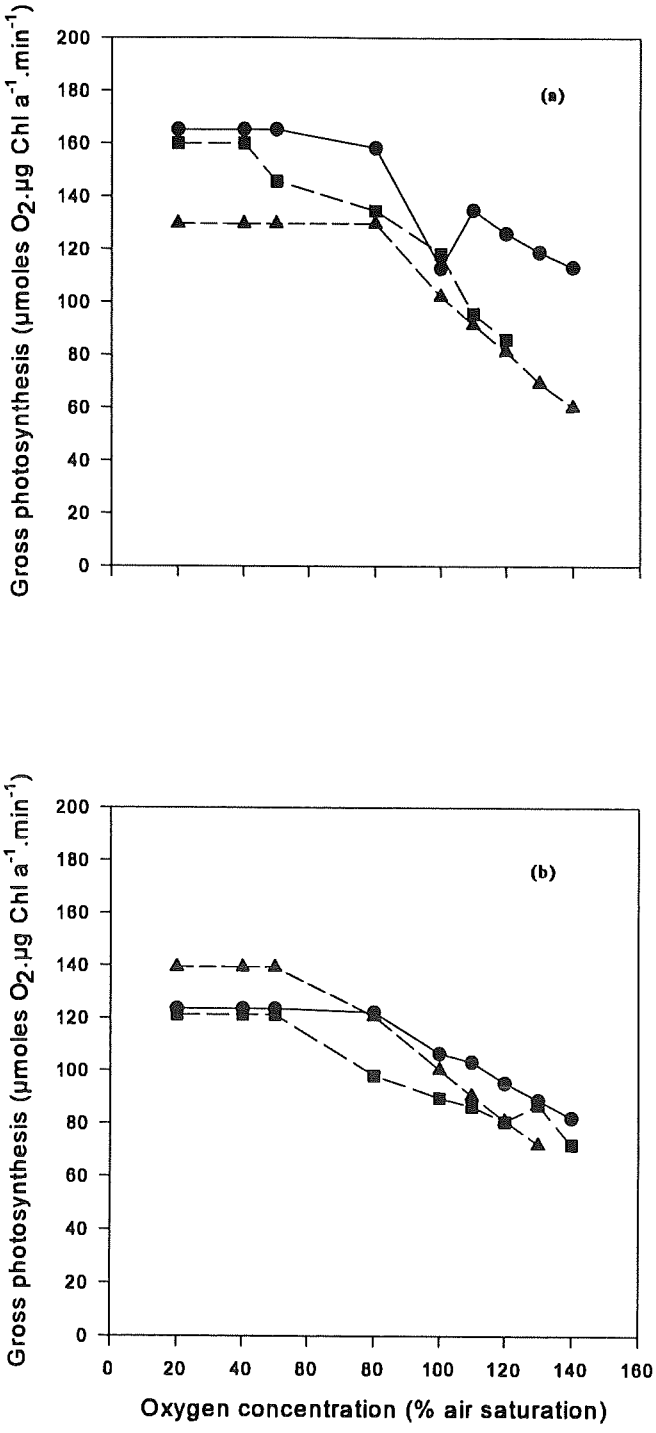

Figure 49 The effect of oxygen concentration on the gross photosynthetic rate of *Skeletonema costatum* at (a) 1200 µmol photons.m^{-2}.sec^{-1}, (b) 2500 µmol photons.m^{-2}.sec^{-1} and three temperatures. (●) 20°C (36.6 µg Chl *a*); (■) 23° C (38.9 µg Chl *a*), (▲) 26°C (44.3 µg Chl *a*).

Increasing temperature at 2500 µmol photons.m^{-2}.sec^{-1} had a similar effect on the O_2 inhibition rate to that seen in *T. chuii* under the same conditions. The O_2 inhibition rate reached a minimum at 23°C and was highest at 26 C and (**Table 6**). Increasing temperature at this irradiance had a different effect on the temperature specific maximum gross photosynthetic rate than at 1200 µmol photons.m^{-2}.sec^{-1} with a maximum at 26°C and a minimum at 23°C.

Increasing irradiance decreased the temperature specific maximum gross photosynthetic rate for all temperatures except at 26°C for which it was increased (**Figure 49**).

Table 6 The effect of irradiance and temperature on the O_2 inhibition rate of 4 species of microalgae.

Species	Irradiance (µmol photons. m^{-2}.sec^{-1})	Inhibition at 20°C (µmoles.µg Chl a^{-1}. min^{-1} per % sat_{air})	Inhibition at 23°C (µmoles.µg Chl a^{-1}. min^{-1} per % sat_{air})	Inhibition at 26°C (µmoles.µg Chl a^{-1}. min^{-1} per % sat_{air})
I. galbana	1200	-0.87	-1.53	-0.84
	2500	-0.46	-0.83	-1.98
T. chuii	1200	-2.78	-3.78	-3.96
	2500	-2.69	-0.44	-2.45
T. suecica	1200	-1.22	-2.52	-3.52
	2500	-1.60	-1.17	-0.61
S. costatum	1200	-0.75	-1.08	-1.15
	2500	-0.66	-0.43	-0.97

Tube length

The effect of tube length was examined in two experiments using specially modified BIOCOIL designs. In the first of these BIOCOILs (BR1E) could be operated at three distinct tube lengths in the photostage (i.e. 26, 56 and 72m) (**Figure 50**). The second modified BIOCOIL (BR2E) could also be operated at the same three total tube lengths in the photostage as in BR1E, however this was achieved by operating the sections of tubing arranged in parallel using a manifold, rather than in series (**Figure 51**).

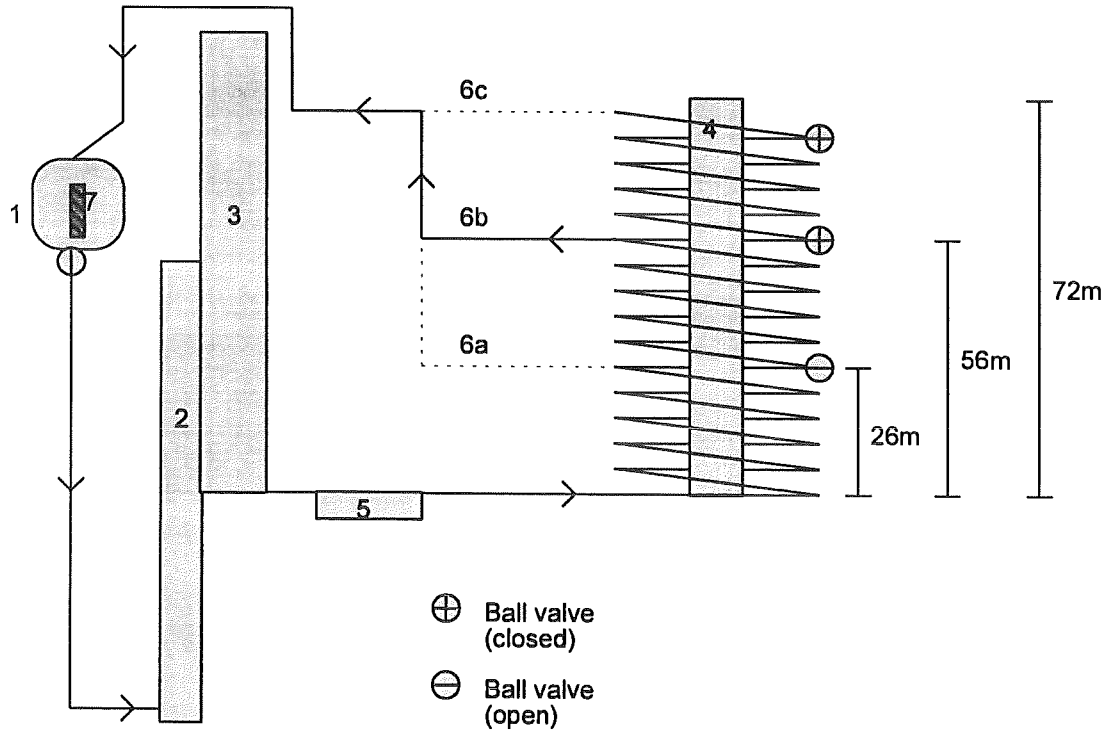

Figure 50 Schematic diagram of BR1E as operated at 56m photostage tube length. 1 = 20L buffer vessel; 2 = airlift; 3 = degasser; 4 = internal lights; 5 = oxygen electrode; 6a = 26m bypass tube; 6b = 56m bypass tube; 6c = 72 m bypass tube; 7 = heat exchange coil in buffer vessel.

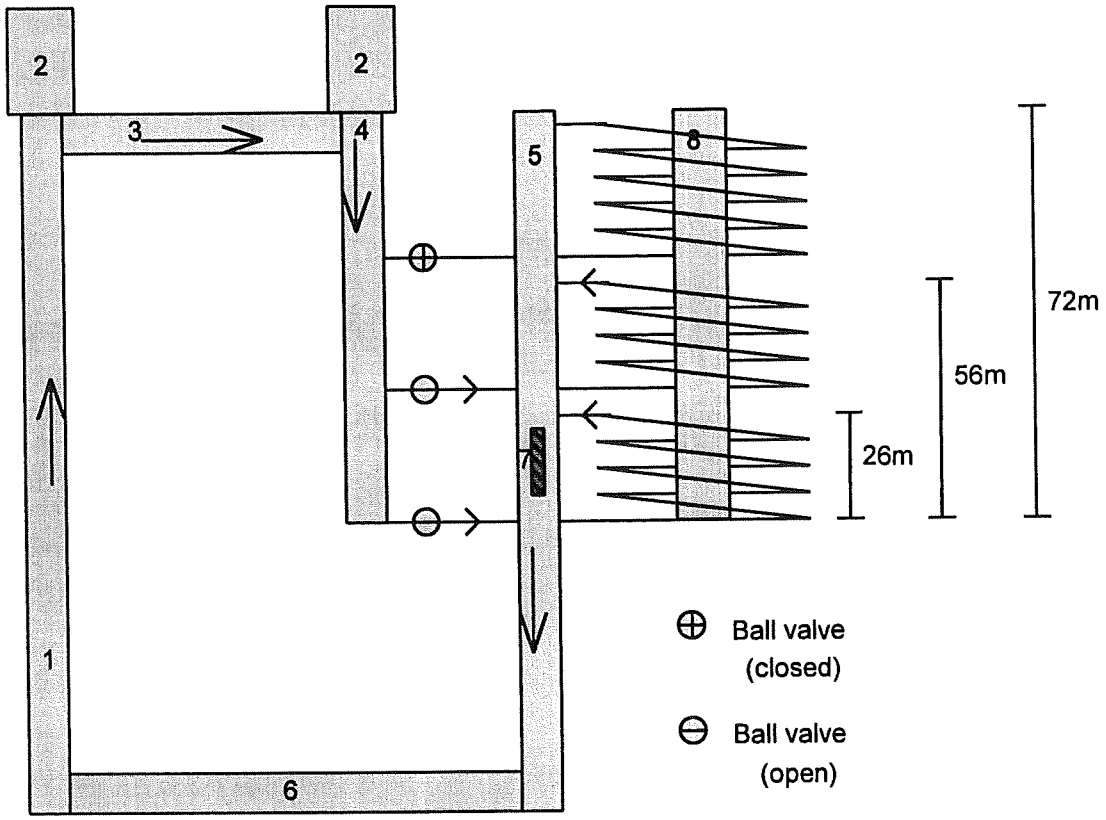

Figure 51 Schematic diagram of BR2E reactor operated at the 56m tube length. 1 = airlift; 2 = degasser; 3 = airlift/manifold connector; 4 = inlet manifold; 5 = outlet manifold; 6 = manifold/airlift return; 7 = heat exchange coil in outlet manifold; 7 - internal lights.

Growth

The growth of *T. chuii* over 15 days of culture followed that of a typical batch culture for all three tube lengths tested (26 m, 56 m and 72 m). The resulting growth curves are shown in **Figure 52**. Growth rate at all three tube lengths was exponential for the first 6 days. Growth levelled of at a maximum after day 7 for the 26 and 56 m lengths, but continued to rise slowly for the 72 m length.

Microscopical analysis of the cells during culture showed that they were smaller at stationary phase in the 72 m length culture than for the other tube length cultures. This smaller size is also borne out by the cell dry weights (**Figure 56**). There was no visible signs of cellular damage (irregular cell shapes, cellular debris etc.) for any of the cultures.

Increasing the tube length of the photostage caused an increase in the maximum cell density of *T. chuii* over the 15 days of growth (**Figure 54b**). Increasing the tube length also increased the specific growth rate (**Figure 54a**). The relationship between tube length and specific growth rate was non-linear with rising from 0.01 h^{-1} for the 26 m length to 0.031 h^{-1} at the 72 m length. The increase in the mean maximum cell number was also non-linear rising from 149.5 ± 6.29 $x10^4$ cells.mL^{-1} at the 26 m tube length to 179.2 ± 3.75 $x10^4$cells.mL^{-1} at the 56 m tube length and 317.5

± 18.2 x10^4 cells.mL^{-1} for the 72 m tube length. There was a statistically significant increase in the mean maximum cell number with each increase in tube length (One Way ANOVA, P <<<0.001).

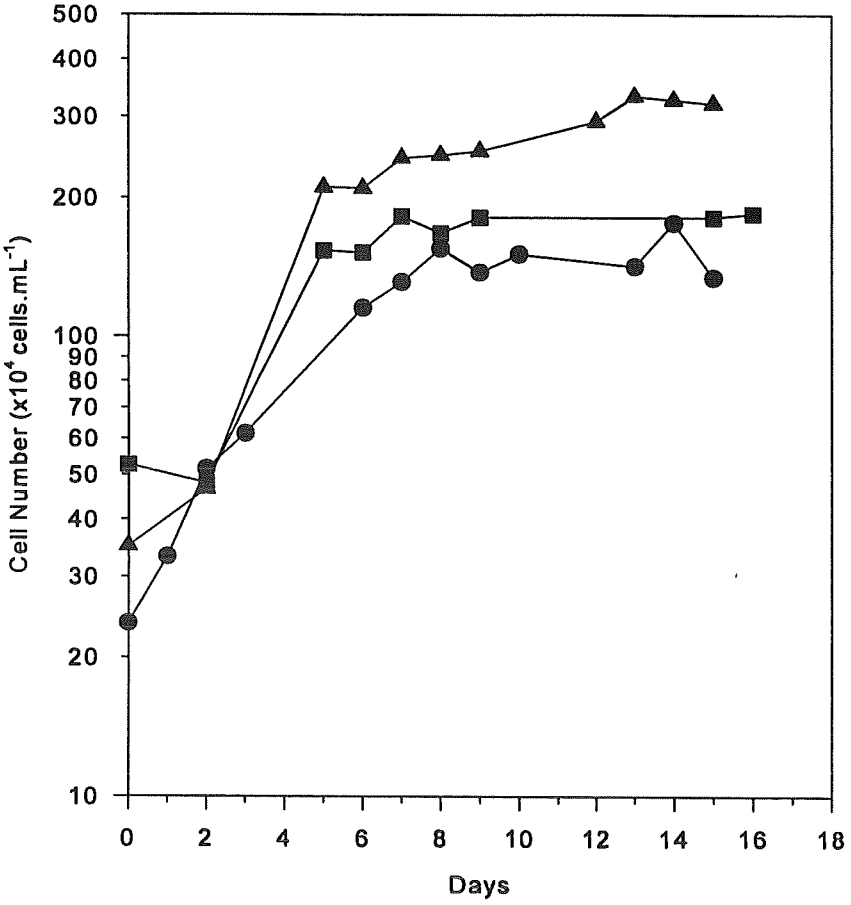

Figure 52 The effect of tube length on the cell density of *Tetraselmis chuii* in BR1E. The culture was grown at 22.6 ± 0.4°C and at a flow rate of 0.336 ± 0.04 m.s^{-1}. (●) 26 m, (■) 56 m, (▲) 72 m.

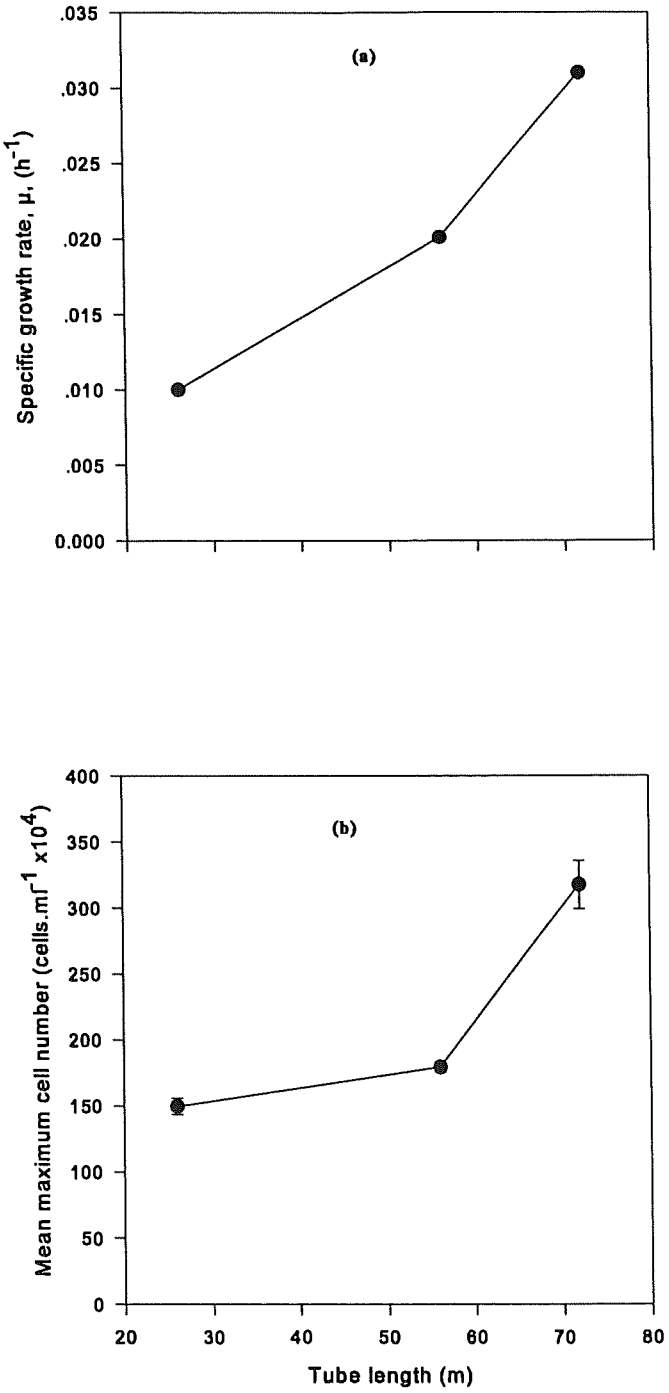

Figure 54 The effect tube length on: (a) the specific growth rate and (b) the mean maximum cell number of *Tetraselmis chuii* grown in Reactor 1 for 15 days at an mean temperature of 22.6 ± 0.4°C and at a flow rate of 0.336 ± 0.04 $m.s^{-1}$. Error bars denote standard error.

Increasing % illuminated volume also increased the gross photosynthetic rate (GPR) of the *T. chuii* culture (**Figure 55**). This increase was non-linear and followed a similar trend as that seen for the μ and the mean maximum cell number (**Figure 54**), with the GPR rising from 92 μmoles.μg Chl a^{-1}.min^{-1} at 25% illuminated volume to 122 μmoles.μg Chl a^{-1}.min^{-1} at 70.6% illuminated volume.

In the longer tubes (i.e. with increasing % illuminated volume) the cells were also smaller and contained less chlorophyll *a* (**Figure 56** and **Figure 57**).

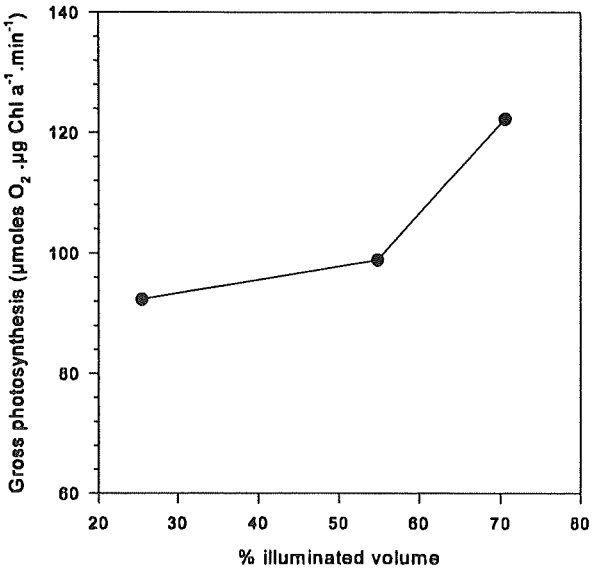

Figure 55 The effect of increasing % illuminated volume on the gross photosynthetic rate of *Tetraselmis chuii* at 22.6 ± 0.4 °C at an irradiance of 50 μmol photons.m^{-2}.s^{-1}.

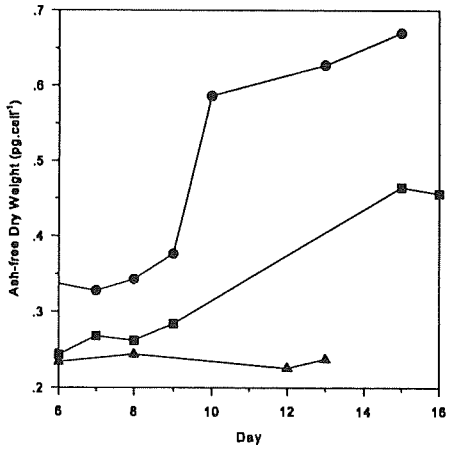

Figure 56 Cell ash-free dry weight of *Tetraselmis chuii* cells growing in BR1E from onset of stationary phase. (●) 26m, (■) 56m, (▲) 72m.

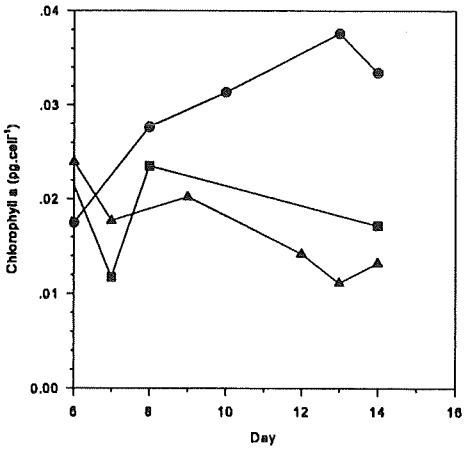

Figure 57 Chlorophyll *a* content of *Tetraselmis chuii* cells growing in BR1E from onset of stationary phase. (●) 26m, (■) 56m, (▲) 72m.

In BR1E the percentage of illuminated volume (vol in tubing / vol airlift) increased linearly with increasing tube length from 25% at 26 m to 70.6% at 72 m. Correspondingly the light/dark ratio increased linearly with increasing % illuminated volume from 4.24 at 25% to 11.76 at 70.6% (**Figure 58**).

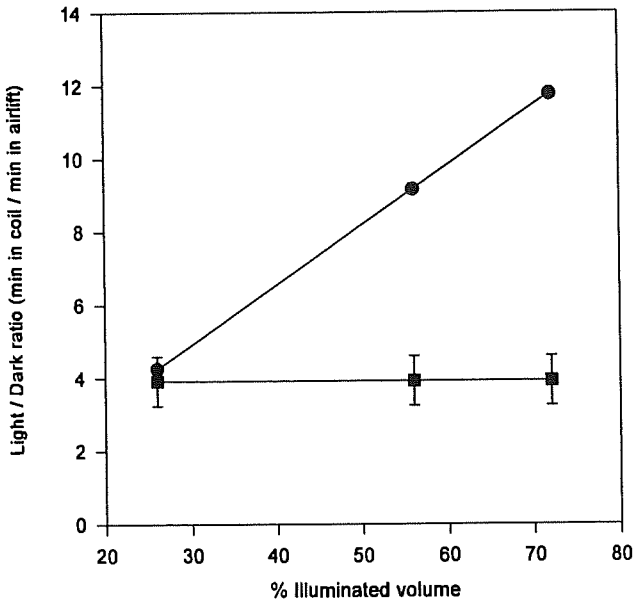

Figure 58 The effect of increasing % illuminated volume on the light/dark ratio in BR1E and BR2E of *Tetraselmis chuii* at 22.6 ± 0.4°C at an irradiance of 50 µmol photons.m^{-2}.s^{1}. (●) BR1E, (■) BR2E (mean ± standard error).

BR2E was constructed to investigate the effect of increasing the % illuminated volume at constant light/dark ratio on the growth of *T. chuii*. This reactor configuration allowed the culture to be exposed to the same increase in % illuminated volume with increasing tube length as in BR1E but with the light/dark ratio held constant (**Figure 58**). BR2E therefore acted as a control for any possible effects associated with increasing light/dark ratio (As opposed to any effects associated solely with % illuminated volume); the light/dark ratio in BR2E was held at an average of 3.9 ± 0.6 for the same % illuminated volumes as in BR1E (**Figure 58**)

The growth of *T. chuii* in Reactor 2 at 56 m was almost identical to that for Reactor 1 at the same tube length (**Figure 59**). The specific growth rate of *T. chuii* in BR2E was only 0.5% less than that

of BR1E and the mean maximum cell density was only 5% greater. These differences were not statistically significant (ANOVA, P = 0.75).

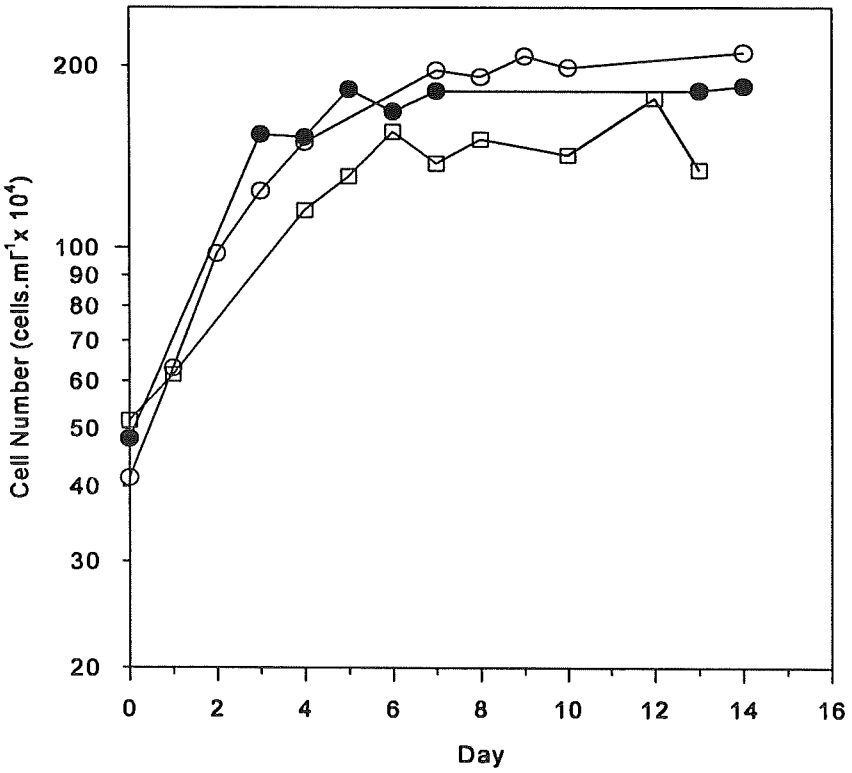

Figure 59 A comparison of the average growth of *Tetraselmis chuii* in BR1E and BR2E at 26 m and 56 m. Both cultures were operated at 22.6 ± 0.4°C and at a flow rate of 0.336 ± 0.04 m.s-1. (○) BR1E, 56 m; (●) BR2E 2, 56 m; (□) BR1E, 26 m

Economic Analysis

A detailed economic analysis is not presented here as this information is commercially sensitive. Biotechna (the industry partner who own the rights to the BIOCOIL) has done a costing based on leasing the BIOCOIL to customers for the production of microalgae for aquaculture. This lease covers the costs of construction and commissioning and ongoing service of the BIOCOIL. Cost estimates are for the production of *Isochrysis galbana* assuming a doubling time of 2 days and operating at a cell density of 1g dry weight.L^{-1}. These assumptions are conservative and given appropriate climatic conditions markedly higher yields can be expected. Furthermore, the cost of constructing the BIOCOILs will decline significantly with experience in constructing them and further improvements in design. The costs are actual lease costs to the customer (i.e. they include a profit component). The costs shown below are for a system constructed in a cyclone-prone area

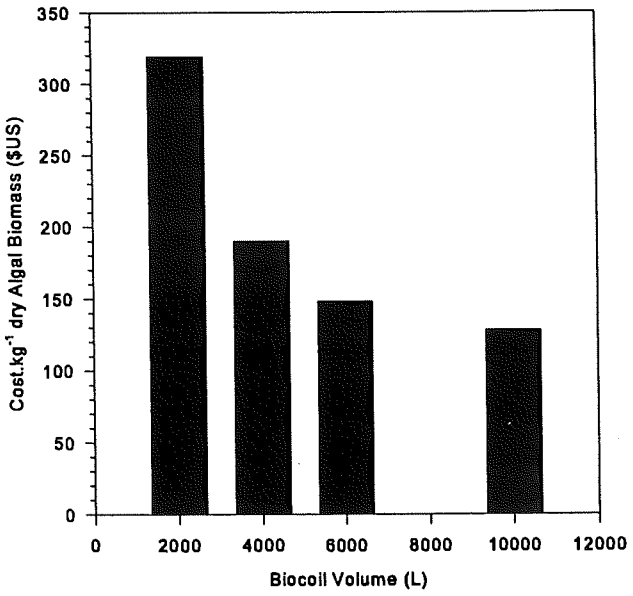

Figure 60 Lease costs for different sized BIOCOILs for the production of 0.5 kg dry weight of *Isochrysis galbana* (T-iso) per 1000L per day. The leasing period is 48 months. Longer leases would be significantly cheaper.

and which therefore requires extensive and costly structural work. If this level of engineering is not required, as would be the case if the BIOCOIL were located in Perth for example, then significant cost-savings of at least 50% can be achieved.

Detailed engineering work we have done in conjunction with the industrial partners in this project indicates that the largest feasible BIOCOIL is 10,000L, although a 5,000L size seems optimal. Figure 59 clearly shows that economics of scale apply to algal production with the BIOCOIL similar to any other algal production systems (see Borowitzka, 1992 for discussion of algal production economics). The algal production costs in the BIOCOIL are similar to those presently in hatcheries (Fulks & Main, 1991; Coutteau & Sorgeloos, 1993; Borowitzka, 1997) and, considering that this culture system is still being optimised, these figures must be seen very much as a maximum benchmark. It also reinforces the fact that small-scale algal culture in hatcheries will always be more expensive than large-scale culture and that in order to obtain cheaper algal feed

for aquaculture a combination of an efficient and reliable large-scale culture system together with the production and use of algal concentrates is essential.

DISCUSSION

During this study we successfully adapted the design of the helical tubular photobioreactor, the BIOCOIL, for culturing microalgae algae for use in aquaculture. The design alterations included the use of airlifts for circulating the algae within the bioreactor, better airlift designs which gave good flow rates but did not cause cell damage, and bioreactor cooling systems using either an internal heat-exchanger (laboratory BIOCOILs) or evaporative cooling (pilot-scale BIOCOILs).

The cultures which grow best in the tubular photobioreactor were *Isochrysis galbana, Tetraselmis suecica, T. chuii, Pavlova lutheri* and *Chroomonas salina.* These species generally achieved higher final cell densities in the outdoor BIOCOILs, presumably because of the higher irradiance. Cell densities > 1g dry weight.L^{-1} were achieved by all of these species and the cultures could be maintained for up to 155 days in semi-continuous culture. The cell densities achieved are generally significantly higher than those achieved in hatcheries and aquaculture facilities, especially if algal culture is carried out indoors in these hatcheries (Lim, 1991; Liao *et al.*, 1993b, Heasman, personal communication and discussions with hatcheries).

The diatom, *Skeletonema costatum* also grew quite well in the laboratory BIOCOILs, however in order to obtain stability of the culture it was found that careful monitoring and regular addition of Si was essential. This species was not tested in the large BIOCOILs outdoors. The other two diatoms, *Chaetoceros gracilis* and *C. calcitrans* proved very difficult to grow and appear to be more shear sensitive. More work on these species is required before they can be considered as candidates for reliable large-scale culture in the BIOCOIL.

Based on the laboratory, glasshouse and pilot plant experiments the daily productivity has been estimated for a 1000L BIOCOIL photobioreactor (see below). Conversion factors from dry weight to cell density can be found in Appendix 3.

Alga	**Calculated production (kg.day^{-1} dry weight)**
Chaetoceros gracilis	0.5 - 0.8
Skeletonema costatum	0.05 -0.1
Pavlova lutheri	0.8 - 0.9
Isochrysis (T-iso)	0.6 - 1.0
Tetraselmis chuii	1.0 - 1.2
Tetraselmis suecica	0.5 - 1.0

With the exception of *Skeletonema costatum*, these productivities are very acceptable, however they need to be confirmed in the pilot-scale reactors. It should also be noted that the data for *C. gracilis* is based on short-term laboratory culture and until this alga can be grown more reliably

and on a larger scale the productivity figure must be considered highly speculative. At this stage it is envisaged that a commercial production unit would have a volume of 5,000 to 10,000 L and would therefore be able to produce about 2.5 to 5 kg dry weight of algae per day. Actual production would, of course, be a little less depending on harvesting efficiencies.

Some of the species initially also showed some sticking to the inside of the tubes of the photobioreactor. However, the sticking could be reduced by adding fresh medium more often and increasing flow rate (turbulence). In normal operational mode for the production of algae for aquaculture the cultures would be harvested regularly and the medium therefore would be replaced more often than was the case in some of these experiments. This would also reduce sticking of the algae.

In the pilot-scale reactors we have also encountered some contamination problems with protozoa, and they required several modifications to the design of the reactors and the operation protocols overcome this problem. Such improvements need to be incorporated in the design of any commercial BIOCOILs.

An important aspect of commercially successful algal culture is to be able to grow the algae in continuous or semi-continuos culture for long periods. This maximises the use of the capital intensive culture system and also reduces labour costs. In this study we have demonstrated that long-term culture of *Isochrysis galbana, Tetraselmis suecica, T. chuii, Pavlova lutheri* and *Chroomonas salina* in the BIOCOIL is possible. In all cases the cultures were stable for at least two months and generally longer than that. The maximum length that a culture can be maintained remains to be determined as the cultures were generally terminated after 2 to 3 months so that other species could be examined. The maximum amount of time a culture can be maintained is not known.

The design of the BIOCOIL also is well suited for computer controlled automation and this is likely to lead to even greater stability of the system and would allow further optimisation of productivity, thus reducing costs.

This early failures of the compressor in the first experiments in the pilot-scale BIOCOILs also highlighted the fact that the algal cultures were actually quite robust and that problems such as compressor failure resulting in a lack of circulation of the culture did not mean death of the culture. This is important from an operational point of view as such failures either due to equipment breakdown or power failures etc. can occur at times, but that they do not mean that algal production necessarily ceases until the reactor has been drained and reinoculated.

The economics of culturing algae in the BIOCOIL are favourable and one unit is to be installed at a mollusc hatchery at the Harbour Branch Oceanographic Institution, Florida, USA. This unit is a 2000L BIOCOIL and is designed to produce at least 1 kg dry wt.day^{-1} of *Isochrysis*. As the design is still new and experience in commercial cultivation is very limited the overall cost of the BIOCOIL is still high. However, with more experience in design, manufacture and operation of these units the cost will fall significantly. As with all microalgal culture systems economics of scale play an important part (see also Borowitzka, 1992). In order to reduce the production cost of the algal biomass the production units should be as large as possible. Furthermore, locating the units at a location with optimal climatic conditions will also reduce operating costs (i.e. additional heating or cooling will not be required). Much of the operation of the BIOCOILs can be automated, thus reducing labour costs. A large production facility is also better able to cope with the likely

need for several species. This strategy however requires the ability to harvest, store and ship the algae to where they are going to be used. The cost of shipping algal concentrates is insignificant compared to the cost of algal production. This second part of this study on algal concentrates (FRDC 93/123 and FRDC 96/342) addresses the question of algal concentrates.

The pilot-scale reactors not only tested the ability of the algal cultures to grow, but also provided us with an opportunity to test materials and equipment so that robust and reliable production units can be designed and constructed. This is also a critical to the successful application of these bioreactors.

REFERENCES

AQUACOP (1977) Elevage larvaire de peneides en millieu tropical. *Act. colloq. CNEXO* **4**: 179-191.

Beardall J. (1989) Photosynthesis and photorespiration in marine phytoplankton. *Aquat. Bot.* **34**: 105-130.

Beidenbach J.M., Smith L. & Lawrence A.L. (1990) Use of a new spray-dried algal product in paneid larviculture. *Aquacult.* **86**: 249-257.

Ben-Amotz A. & Avron M. (1989) The biotechnology of mass culturing *Dunaliella* for products of commercial interest. In: Cresswell R.C., Rees T.A.V. & Shah N. (eds.), *Algal and cyanobacterial biotechnology*. Longman Scientific & *Technical: Harlow. pp. 91-114.*

Borowitzka M.A. (1991) Algal Biotechnology Products and Processes - Matching Science and Economics. *J. Phycol., Suppl.* **27**: 10.

Borowitzka M.A. (1992) Algal biotechnology products and processes: Matching science and economics. *J. Appl. Phycol.* **4**: 267-279.

Borowitzka M.A. (1996) Closed algal photobioreactors: design considerations for large-scale systems. *J. mar. biotechnol.* **4**: 185-191.

Borowitzka M.A. (1997) Algae for aquaculture: Opportunities and constraints. *J. Appl. Phycol.* **9**: 393-401..

Brown A. (1972) Experimental techniques for preserving diatoms used as food for larval *Panaeus aztecus. Proc. Nat. Shellfisheries Ass.* **62**: 21-25.

Brown M.R. (1991) The amino-acid and sugar composition of 16 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **145**: 79-99.

Brown M.R. (1995) Effects of storage and processing on the ascorbic acid content of concentrates prepared from *Chaetoceros calcitrans. J. Appl. Phycol.* **7**: 495-500.

Brown M.R. & Farmer C.L. (1994) Riboflavin content of 6 species of microalgae used in mariculture. *J. Appl. Phycol.* **6**: 61-65.

Brown M.R. & Jeffrey S.W. (1992) Biochemical composition of microalgae from the green algal Classes Chlorophyceae and Prasinophyceae .1. Amino acids, sugars and pigments. *J. Exp. Mar. Biol. Ecol.* **161**: 91-113.

Brown M.R. & Miller K.A. (1992) The ascorbic acid content of 11 species of microalgae used in mariculture. *J. Appl. Phycol.* **4**: 205-215.

Brown M.R., Jeffrey S.W. & Garland C.D. (1989) Nutritional aspects of microalgae used in mariculture; a literature review. *CSIRO Marine Laboratories Report* **205**: 1-43.

Brown M.R., Garland C.D., Jeffrey S.W., Jameson I.D. & Leroi J.M. (1993) The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp (Clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. *J. Appl. Phycol.* **5**: 285-296.

Canzonier W.J. & Brunetti R. (1976) Low-cost continuous algal culture system. In: Persoone G. & Jaspers E. (eds.), *Proceedings of the 10thEuropean Symposium on Marine Biology* 1. Universa Press: Wetteren. pp. 27-31.

Carpenter J.H. (1966) New measurements of oxygen solubility in pure and natural waters. *Limnol. Oceanogr.* **11**: 264-277.

Chaumont D. (1993) Biotechnology of algal biomass production - A review of systems for outdoor mass culture. *J. Appl. Phycol.* **5**: 593-604.

Chaumont D., Thepenier C., Gudin C. & Junjas C. (1988) Scaling up a tubular photoreactor for continuous culture of *Porphyridium cruentum* from laboratory to pilot plant (1981 - 1987). In: Stadler T., Mollion J., Verdus M.C., Karamanos Y., Morvan H. & Christiaen D. (eds.), *Algal Biotechnology*. Elsevier Applied Science: London. pp. 199-208.

Chrismadha T. & Borowitzka M.A. (1994) Effect of cell density and irradiance on growth, proximate composition and eicosapentaenoic acid production of *Phaeodactylum tricornutum* grown in a tubular photobioreactor. *J. Appl. Phycol.* **6**: 67-74.

Costa-Pierce B.A. (1982) Construction and use of free-standing solar silos as combined mass algal/fish culture units. *Aquacult. Eng.* **1**: 239-244.

Coutteau P. & Sorgeloos P. (1992) The use of algal substitutes and the requirement for live algae in the hatchery and nursery of bivalve molluscs: an international survey. *J. Shellfish. Res.* **11**: 467-476.

Coutteau P. & Sorgeloos P. (1993) Substitute diets for live algae in the intensive rearing of bivalve molluscs - a state of the art report. *World Aquaculture* **24**: 45-52.

Curatolo A., Ryan M.J. & Mercer J.P. (1993) An Evaluation of the performance of Manila Clam spat (*Tapes philippinarum*) fed on different rations of spray-dried algae (*Tetraselmis suecica*). *Aquacult.* **112**: 179-186.

De Pauw N. & Persoone G. (1988) Micro-algae for aquaculture. In: Borowitzka M.A. & Borowitzka L.J. (eds.), *Micro-algal Biotechnology*. Cambridge University Press: Cambridge. pp. 197-221.

Deroeck-Holtzhauer Y., Claire C., Bresdin F., Amicel L. & Derrien A. (1993) Vitamin, free amino acid and fatty acid compositions of some marine planktonic microalgae used in aquaculture. *Bot. Mar.* **36**: 321-325.

Dunstan G.A., Volkman J.K., Jeffrey S.W. & Barrett S.M. (1992) Biochemical composition of microalgae from the green algal Classes Chlorophyceae and Prasinophyceae .2. Lipid classes and fatty acids. *J. Exp. Mar. Biol. Ecol.* **161**: 115-134.

Dunstan G.A., Volkman J.K., Barrett S.M., Leroi J.M. & Jeffrey S.W. (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochem.* **35**: 155-161.

Epifanio C.E. (1979a) Comparison of yeast and algal diets for bivalve molluscs. *Aquacult.* **16**: 187-192.

Epifanio C.E. (1979b) Growth in bivalve molluscs: nutritional effect of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* and the hard clam *Mercenaria mercenaria*. *Aquacult.* **18**: 1-12.

Fox J.M. (1983) Intensive algal culture techniques. In: McVey J.P. (ed.), *CRC Handbook of Mariculture. Crustacean Aquaculture* 1. CRC Press, Inc.: Boca Raton, Florida. pp. 15-41.

Fulks W. & Main K.L. (1991) The design and operation of commercial-scale live feeds production systems. In: Fulks W. & Main K.L. (eds.), *Rotifer and Microalgae Culture Systems*. The Oceanic Institute: Honolulu. pp. 3-52.

Gallardo P.P., Alfonso E., Gaxiola G., Soto L.A. & Rosas C. (1995) Feeding schedule for *Penaeus setiferus* larvae based on diatoms (*Chaetoceros ceratosporum*), flagellates (*Tetraselmis chuii*) and *Artemia* nauplii. *Aquacult.* **131**: 239-252.

Griffith G.W., Murphy Kenslow M.A. & Ross L.A. (1973) A mass culture method for *Tetraselmis* sp - A promising food for larval crustaceans. *Proc. Ann. Meet. World Maricult. Soc.* **4**: 289-294.

Guillard R.R.L. & Ryther J.H. (1962) Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **8**: 229-239.

Hidu H. & Ukeles R. (1962) Dried unicellular algae as food for the larvae of the hard shell clam, *Mercenaria mercenaria*. *Proceedings, National Shellfish Association* **53**: 85-101.

Hu Q., Guterman H. & Richmond A. (1996) A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnol. Bioeng.* **51**: 51-60.

Jeffrey S.W. & Humphrey G.F. (1975) New spectrophotometric equations for determining chlorophylls *a*, *b*, *c1* and *c2* in higher plants, algae and natural populations. *Biochem. Physiol. Pflanzen* **167**: 191-194.

Laing I. & Millican P.F. (1992) Indoor nursery cultivation of juvenile bivalve molluscs using diets of dried algae. *Aquacult.* **102**: 231-243.

Laing I. & Verdugo C.G. (1991) Nutritional value of spray-dried *Tetraselmis suecica* for juvenile bivalves. *Aquacult.* **92**: 207-218.

Laing I., Child A.R. & Janke A. (1990) Nutritional value of dried algae diets for larvae of Manila Clam (*Tapes philippinarum*). *J. Mar. Biol. Ass. U.K.* **70**: 1-12.

Lee C.S. & Tamaru C.S. (1993) Live larval food production at the Oceanic Institute, Hawaii. In: McVey J.P. (ed.), *CRC Hadbook of Mariculture. Crustacean Aquaculture* 1. CRC Press: Boca Raton. pp. 15-28.

Liao I.C., Su H.M. & Lin H.H. (1993a) Larval foods for penaeid prawns. In: McVey J.P. (ed.), *CRC Handbook of Mariculture. Crustacean Aquaculture* 1. CRC Press: Boca Raton, Florida. pp. 29-59.

Liao I.C., Su H.M. & Lin J.H. (1993b) Larval foods for paneid prawns. In: McVey J.P. (ed.), *CRC Handbook of Mariculture* 1. Crustacean Aquaculture. CRC Press: Boca Raton. pp. 29-59.

Lim L.C. (1991) An overview of live feeds production systems in Singapore. In: Fulks W. & Main K.L. (eds.), *Rotifer and Microalgae Culture Systems*. The Oceanic Institute: Hololulu. pp. 203-220.

Millamena O.M., Aujero E.J. & Borlongan I.G. (1990) Techniques on algae harvesting and preservation for use in culture and as larval food. *Aquacult. Eng.* **9**: 295-304.

Mock C.R. (1972) Larval culture of penaeid shrimp at the Galveston Biological Laboratory. *NOAA Technical Report* **NMFS CIRC-388**: 33-40.

Mock C.R. & Murphy M.A. (1970) Techniques for raising penaeid shrimp from the egg to post-larvae. *Proceedings First Annual Workshop, World Mariculture Society* **1**: 143-158.

Montaini E., Zittelli G.C., Tredici M.R., Grima E.M., Sevilla J.M.F. & Perez J.A.S. (1995) Long-term preservation of *Tetraselmis suecica* - influence of storage on viability and fatty acid profile. *Aquacult.* **134**: 81-90.

Nell J.A. & O'Connor W.A. (1991) The evaluation of fresh algae and stored algal concentrates as a food source for Sydney Rock Oyster, *Saccostrea commercialis* (Iredale and Roughley), larvae. *Aquacult.* **99**: 277-284.

Numaguchi K. & Nell J.A. (1991) Effects of gelatin acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney Rock Oyster, *Saccostrea commercialis* (Iredale and Roughley), larvae. *Aquacult.* **94**: 65-78.

O'Connor W.A. & Nell J.A. (1992) The potential of algal concentrates for the production of Australian bivalves. In: Allan G.L. & Dall W. (eds.), *Proceedings, Aquaculture Nutrition Workshop, Salamander Bay, April 1991*. NSW Fisheries, Brackish Water Fish Culture Research Station: Salamander Bay, NSW. pp. 200-201.

O'Meley C. & Daintith M. (1993) *Algal cultures for marine hatcheries.* Aquaculture Sourcebooks, Launceston, 35 pp.

Oswald W.J. (1988) Large-scale algal culture systems (engineering aspects). In: Borowitzka M.A. & Borowitzka L.J. (eds.), *Micro-Algal Biotechnology*. Cambridge University Press: Cambridge. pp. 357-394.

Pulz O. (1994) Open-air and semi-closed cultivation systems for the mass cultivation of micro-algae. In: Phang S.M., Lee K., Borowitzka M.A. & Whitton B. (eds.), *Algal Biotechnology in the Asia-Pacific Region*. Institute of Advanced Studies, University of Malaya: Kuala Lumpur. pp. 113-117.

Richmond A., Vonshak A. & Arad S. (1980) Environmental limitations in outdoor production of algal biomass. In: Shelef G. & Soeder C.J. (eds.), *Algae Biomass*. Elsevier/North Holland Biomedical Press: Amsterdam. pp. 65-72.

Richmond A., Lichtenberg E., Stahl B. & Vonshak A. (1990) Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways. *J. Appl. Phycol.* **2**: 195-206.

Richmond A., Boussiba S., Vonshak A. & Kopel R. (1993) A new tubular reactor for mass production of microalgae outdoors. *J. Appl. Phycol.* **5**: 327-332.

Robinson L.F. & Morrison A.W. (1992) Biomass production apparatus. USA Patent Number 5,137,828.

Robinson L.F., Morrison A.W. & Bamforth M.R. (1988) Improvements relating to biosynthesis. European Patent Number 261,872.

Sato V. (1991) The development of a phytoplankton production system as a support base for finfish larval rearing research. In: Fulks W. & Main K.L. (eds.), *Rotifer and Microalgae Culture Systems*. The Oceanic Institute: Hololulu. pp. 257-273.

Tanticharoen M., Bunnag B. & Vonshak A. (1993) Cultivation of *Spirulina* using secondary treated starch wastewater. *Australas. Biotechnol.* **3**: 223-226.

Tredici M.R. & Materassi R. (1992) From open ponds to vertical alveolar panels - The Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *J. Appl. Phycol.* **4**: 221-231.

Venkataraman L.V. & Becker E.W. (1985) *Biotechnology and utilization of algae - The Indian experience*. Department of Science & Technology, New Delhi, 257 pp.

Volkman J.K., Smith D.J., Eglinton G., Forsberg T.E.V. & Corner E.D.S. (1981) Sterol and fatty acid composition of four marine haptophycean algae. *J. Mar. Biol. Ass. U.K.* **61**: 509-527.

Volkman J.K., Jeffrey S.W., Nichols P.D., Rogers G.I. & Garland C.D. (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **128**: 219-240.

Volkman J.K., Dunstan G.A., Jeffrey S.W. & Kearney P.S. (1991) Fatty acids from microalgae of the genus *Pavlova*. *Phytochem.* **30**: 1855-1859.

Volkman J.K., Brown M.R., Dunstan G.A. & Jeffrey S.W. (1993) The biochemical composition of marine microalgae from the Class Eustigmatophyceae. *J. Phycol.* **29**: 69-78.

Vonshak A., Abeliovich A., Boussiba S., Arad S. & Richmond A. (1982) Production of *Spirulina* biomass: effects of environmental factors and population density. *Biomass* **2**: 175-185.

Watanabe Y. & Hall D.O. (1996) Photosynthetic production of the filamentous cyanobacterium *Spirulina platensis* in a cone shaped helical tubular photobioreactor. *Appl. Microbiol. Biotech.* **44**: 693-698.

Watson R.H., Jones G.G. & Jones B.L. (1986) Using centrifuged algae for feeding oyster larvae. *J. Shellfish. Res.* **5**: 136.

APPENDIX 1: INTELLECTUAL PROPERTY

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The BIOCOIL design is covered by several patents (Robinson *et al.*, 1988; Robinson & Morrison, 1992) owned by Biotechna Environmental Ltd. This project has developed several significant improvements in the design and construction of the BIOCOIL to make it suitable for culturing the relatively fragile marine algal species used in aquaculture both indoors and outdoors. Methods for culturing the algae, optimising cell yield and monitoring culture health have also been developed. These improvements have created a reliable, easy to operate and productive algal production system.

Publications and abstracts arising wholly or in part from this study:

Borowitzka M.A. (1996) Closed algal photobioreactors: design considerations for large-scale systems. *Journal of Marine Biotechnology* **4**: 185-191.

Borowitzka M.A. (1997) Algae for aquaculture: Opportunities and constraints. *Journal of Applied Phycology* **9**: 393-401.

Borowitzka M.A. (1998a) Appropriate technology in large-scale algal culture systems. In: de Paula E.J., Corediro-Marino M., Santos D.P., Plastino E.M., Fujii M.T. & Yokoya N.S. (eds.), *Anais do IV Congresso latino-Americano, II reuniao Iberio-Americana, VII Reuniao Brasileira, de Ficologia* II. Sociedade Ficologica de America Latina e Caribe & Sociatade Brasiliera de Ficologia: Caxambu. pp. 1-12.

Borowitzka M.A. (1998b) Tubular photobioreactors for large-scale algal culture. In: Subramanian G., Kaushik B.D. & Venkatamaran G.S. (eds.), *Cyanobacterial biotechnology*. Oxford & IBH Publishing Co: New Delhi. pp. 249-257.

Borowitzka M.A. (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* **70**: 313-321.

Borowitzka, M.A. (1999) A helical tubular photobioreactor for large-scale culture of microalgae for aquaculture and other applications. Abstracts. World Aquaculture '99, (26 April-2 May, Sydney). page 88.

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APPENDIX 3: Dry Weight to Cell Number Conversion

This data is based on means of regular measurements of ash-free dry weights (i.e. organic weight only) and cells densities over a range of culture conditions and serve as a guide only. The weight per cell can change significantly under different culture conditions.

Species	Weight per cell ($g.cell^{-1}$)	No of cells per 1g dry weight	Equivalent culture volume for 1 g dry weight at a cell density of $1x10^6$ $cells.mL^{-1}$
Isochrysis (T-iso)	$1.0x10^{-10}$	$1.0x10^{10}$	1L
Tetraselmis chuii	$2.77x10^{-10}$	$3.6x10^{9}$	3.6L
Chaetoceros gracilis	$3.63x10^{-10}$	$2.75x10^{9}$	2.75L
Pavlova lutheri	$4.0x10^{-11}$	$2.5x10^{11}$	25L