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PHYSIOLOGICAL AND GROWTH RESPONSES OF *Chlorella vulgaris* AND *Scenedesmus subspicatus* TO A RANGE OF ENVIRONMENTAL FACTORS

by

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE AND MATHS
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PHYSIOLOGICAL AND GROWTH RESPONSES OF *Chlorella vulgaris* AND *Scenedesmus subspicatus* TO A RANGE OF ENVIRONMENTAL FACTORS

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This research provides an advanced understanding of the physiological responses of two algal species, *Chlorella vulgaris* and *Scenedesmus subspicatus* (Chlorophyta) to changing environmental conditions likely to occur in Waste Stabilisation Ponds (WSPs).

Algal growth in relation to the three key nutrients carbon, nitrogen and phosphorus was studied in batch cultures. Maximum growth rates and half saturation constants for nutrient-limiting growth were obtained. Additional studies were conducted on rates of nutrient uptake. Growth characteristics in relation to light and temperature were obtained.

Bicarbonate was found to be a suitable carbon source for both species. Maximum growth rates of around 0.07 h^{-1} were achieved at concentrations of around 10 mmol C l^{-1}. The results showed that nitrate uptake became increasingly light dependent with increases in temperature. Phosphate was taken up from the medium in excess of immediate growth requirements and uptake rates did not show light or temperature dependence at the range of concentrations used. Respiration rates were shown to depend on light and temperature. Photosynthetic activity was registered at light intensities as low as 7.8 μmol $m^{-2}s^{-1}$ and 5 °C of temperature. Both light and temperature affected growth rates. Both species reached maximum light-specific growth rates at 47.0 μmol $m^{-2}s^{-1}$ at all temperatures tested. Below 15 °C *C. vulgaris* showed higher growth rates than *S. subspicatus*, but this was reversed at 20 °C.

Experiments on survival showed that a proportion of cells of *C. vulgaris* could survive long periods (22 weeks) of dormancy in complete darkness and low temperatures (+4 and –20 °C). Despite a hardening procedure, *S.subspicatus* showed no survival after exposure to –20 °C and limited ability to resume growth and carry out photosynthetic oxygen production after exposure to +4 °C and complete darkness up to 14-15 weeks. Overall the results indicate that *C. vulgaris* is better adapted to growth at low temperature and light intensities, but may be out-competed by *S. subspicatus* in warmer brighter conditions.

The study aimed to contribute to a quantitative understanding of factors affecting the growth, photosynthetic oxygen production and nutrient uptake rates of microalgae. These results could be of use in the design of cold climate WSPs.

TO MY MUM, WITH ALL MY LOVE

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Chapter 1 INTRODUCTION

1.1 Waste Stabilisation Ponds as a treatment system

A Waste Stabilisation Pond (WSP) is a low-cost method for the biological stabilisation of wastewater. It uses a system of shallow ponds in which treatment is mainly effected by a mutualistic consortium of algae and bacteria. The aerobic degradation of organic materials in the wastewater is carried out by bacteria using oxygen produced photosynthetically by the algae which take up nutrients and trace elements. WSPs are in common usage worldwide and cover a range of climatic zones from tropical to high latitude cold climate regions.

The interest in the use of algae to treat wastewater arose because of the potential advantages compared to conventional treatment processes (Bush *et al.* 1963; Talbot & Noüe 1993; Craggs *et al.* 1996):

1) long retention times offering a high degree of process stability and pathogen removal;

2) high levels of nutrient removal;

3) low cost;

4) minimal use of energy and chemicals.

The use of algae means that WSPs are a natural self-purification system that can be controlled and optimised by adopting an appropriate engineering design (Oswald 1957). WSPs systems have been proven as an excellent method of wastewater treatment due to high effluent quality, low cost and simple operation (Mara & Pearson 1998).

A widely used classification system for types of WSP based on conditions within the pond environment is as follows (Middlebrooks *et al.* 1982):

- Anaerobic (with prevailing anaerobic conditions within the pond, the pond is designed for high loading).

- Facultative (with coexistence of predominantly anaerobic conditions at the pond bottom and predominantly aerobic conditions at the surface with equilibration of both in water column).

- Aerobic (with aerobic conditions prevailing in the pond, the pond is designed to maximise the impact of algae on wastewater).

Numerous types of WSP system have been developed since the major advantages of this method of wastewater treatment were realised (Marais 1974; Middlebrooks *et al.* 1982; Mara 1987).

Despite the advances in modern WSP design, the fundamental basis for waste stabilisation remains as an algal-bacterial interaction. The algal contribution to wastewater treatment is generally considered to be as follows:

- providing oxygen for efficient biodegradation of organic matter,

- participation in the process of ammonia nitrogen removal, and

- reducing pathogen bacteria contamination of final effluent.

Algae also contribute to enhancing sedimentation and disinfection and in the removal of nutrients, heavy metal and toxic organics. However, the mechanisms for these contributions are not well understood.

While traditional WSP design considers BOD (biochemical oxygen demand) loading, rates of pathogen removal, type of waste to be treated or quality of effluent to achieve, the presence and well being of the WSP algal population is taken for granted. This is due to the speed with which algae appear and then thrive in wastewater, especially in warm climates. Successful wastewater treatment can only occur in the systems with actively growing and propagating algal population, however, and therefore an understanding of the mechanisms that control algal growth and population is essential for effective and efficient wastewater treatment. For example problems in wastewater treatment in colder climates, such as poor quality effluent, can be attributed to the lack

of satisfactory knowledge of algal growth and activities such as oxygen production and nutrients removal, during the transition periods between seasons.

In summary, WSP provides an economic and environmentally sound method of treating wastewater. Like most wastewater treatment processes WSPs have been developed empirically and it is only in recent years that scientific research has been applied to increasing our understanding of these systems, with a view to future improvements in design and performance. Tropical and temperate ponds have received far more attention to date than have cold or continental climate systems, and the current research is primarily aimed at the latter.

1.2 Aim and objectives of the research

The principle aim of the research is to develop an improved quantitative understanding of the growth characteristics and activity of selected green algae typical of those found in WSPs. The work is intended to provide a detailed insight into the factors affecting the growth, photosynthetic oxygen production and rates of nutrient uptake. For this purpose the algal species *Scenedesmus subspicatus* and *Chlorella vulgaris*, both members of the family of green algae (Chlorophyceae), will be studied.

To achieve the aim several key parameters have been identified that are of importance to their operation of cold or continental climate WSPs especially during the winter dormancy and spring warm-up period. These include:

- algal activity at suboptimal temperatures;
- algal activity at suboptimal light intensities;
- ability of algae to resume growth after a period of dormancy;
- oxygen production, carbon fixation and nutrient uptake in a range of conditions;
- the influence of changing pH.

To investigate these parameters the following specific objectives were identified:

1) to establish the growth kinetics of algal species in relation to nutrient availability (especially carbon, nitrogen, phosphorus);
2) to establish the influence of the irradiance levels and temperature on exponentially growing pure cultures;
3) to evaluate the effect of low temperature on the level of biomass production and viability of the algal population;
4) to assess the ability of algal cells to resume growth and photosynthetic activity after a period of cold and dark induced dormancy.

Chapter 2 BACKGROUND AND LITERATURE REVIEW

2.1 Wastewater as a medium for algae

2.1.1 Introduction

For successful performance, a waste stabilisation pond should contain an algal population that is active and capable of responding to changing conditions by substantial changes in number and constituent members. Algae found in WSPs are usually restricted to pollution-tolerant species (Palmer 1980). These types can tolerate relatively high concentrations of toxic substances common in wastewater such as NH_3 and S^{2-} (Abeliovich & Azov 1976; Abeliovich 1986; Mills 1987). Another significant feature of algal presence in a pond is that the final products of bacterial waste decomposition, such as ammonia, phosphates and other compounds, are absorbed and assimilated by algae. Furthermore, some algal species are known to use organic molecules for heterotrophic growth. In this way, the living algal cells would consist of relatively stabilised organic components (Palmer 1980).

2.1.2 Nutritional modes of algae

The classification of algae by nutritional mode is complex and not always clear. The uncertainty is due to the ability of many algal species to alter their nutritional mode in response to variation in different factors, such as changes in the chemical composition of media, temperature, or availability of light in their environment (Kaplan *et al.* 1986). It is possible, however, to distinguish between major forms of nutrition: autotrophy and heterotrophy.

The term 'autotrophy' refers to the nutritional mode in which organisms either gain energy from light or from the oxidation of inorganic compounds or ions, and all essential elements from inorganic compounds. Most of algae are phototrophs, i.e. they

obtain energy from absorption of light and electrons for the reduction of carbon dioxide from the oxidation of an inorganic substrate, usually water, with the evolution of oxygen.

The term 'heterotrophy' refers to the nutritional mode in which one species uses organic compounds synthesised by another species for its material and energy needs. In most cases, this type of nutrition relates to bacteria and fungi found in WSPs. The majority of WSP algal species have been found to be capable of employing both modes in their metabolism, however (Abeliovich & Weisman 1978). As already stated, there are a number of factors which may determine algal nutritional modes. For instance, the presence of glucose in a medium was found to stimulate the growth of *Scenedesmus* and *Chlorella* species (Abeliovich 1980). As a source of organic nitrogen, the amides, urea and glutamine were found to be used by both species (Thomas 1968).

Further confirmation that algal species commonly inhabiting wastewater are capable of facultative heterotrophy comes from the observation that, although the high density of suspended solids usually present in a pond (Miller *et al.* 1977) and the strong colour of wastewater may substantially restrict the availability of solar radiation to algal cells, the amount of living algal cells below the photic zone in a WSP is nearly equal to that in the photic zone (Groeneweg *et al.* 1980).

Practical observations in High Rate Ponds (HRPs) in Jerusalem, Israel showed that with a high wastewater biochemical oxygen demand (BOD) of around 700 mg O_2 l^{-1} and a relatively short retention time of 4 days, 90-99% of BOD was successfully removed due to heterotrophically assimilated carbon (Abeliovich & Weisman 1978). The study also showed that between 20 and 50% of algal populations could become heterotrophic, in response to the high concentration of organics in domestic wastewater.

Species that are able to oxidise organic matter or use different energy sources have a valuable advantage in survival in a medium such as wastewater over those species that are obligate autotrophs. Algal heterotrophy has a more occasional character in WSPs, however, for several reasons. Firstly, this is due to the fact that the heterotrophically feeding algal population is forced to compete in space and time with the larger and

metabolically faster bacterial population for substrate availability. Secondly, in the classical design of WSP system, the algal population is mainly present at the stage of the facultative pond, which is fed on the effluent from an anaerobic pond with a vast bacterial population. Thus, the small organic molecules that are appropriate for algal nutrition are also a preferred diet for bacteria, and consequently will be removed from wastewater at the early stage (i.e. in the anaerobic pond), leaving only the negligible amount to escape to the facultative ponds.

From the operational point of view, however, algal heterotrophy is not desirable in WSPs, as this metabolic mode will substantially reduce the amount of photosynthetic oxygen production (Ansa-Asare *et al.* 2000). For successful wastewater treatment in WSPs it is therefore desirable to maintain the pond operation at a state in which the majority of the algal population is phototrophic.

2.1.3 Algal groups

Among the variety of algal species, the group of green algae (Chlorophyta) have attracted particular scientific attention due to their widespread presence in successfully operated WSPs. Palmer (1980) provides a list of algal species commonly found in WSPs, where the majority of species belong to Chlorophyta. Oswald (1988) reported that green algae are the dominant species in WSPs. Other species of algae such as blue-green algae and diatoms may also make a contribution to pond oxygen supply.

Noüe *et al.* (1992) reviewed the possibilities of using blue-green algae in tertiary biological treatment of wastewater and concluded that this group has a valuable potential. The presence of blue-green algae in WSP effluent is not desirable, however, due to the poorly-understood phenomenon of occasional production of toxic substances as the end-products of their metabolism (Gorham 1964; Vinberg *et al.* 1966). In addition large numbers of blue-green algae are present in the water column only temporarily, during a short period of blooming, after which they sink to the bottom and cease active oxygen production.

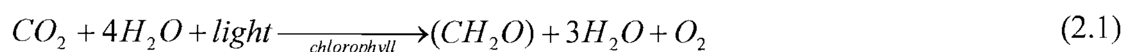
Occasional reports of possible domination by diatoms over the spring season in natural temperate waterbodies suggest that this group may play an important role in aquatic oxygen balance (Oviatt *et al.* 2002). There is insufficient literature, however, on the question of the contribution by diatoms to WSP oxygen supply.

2.2 Photosynthesis and Carbon sources

2.2.1 Photosynthesis

The process by which plants synthesise organic compounds from inorganic raw materials in the presence of sunlight is known as photosynthesis.

The major chemical pathway in photosynthesis is the conversion of carbon dioxide and water to carbohydrates and oxygen. The photosynthetic process is highly complicated, but the general synoptic equation can be written as follows (Hall & Rao 1999):



$$CO_2 + 4H_2O + light \xrightarrow[chlorophyll]{} (CH_2O) + 3H_2O + O_2 \qquad (2.1)$$

The water molecule acts as a hydrogen donor and carbon dioxide as the hydrogen acceptor with an accumulation of 468.9 kJ $mole^{-1}$ of CO_2 reduced. Thus the reaction products carbohydrates and oxygen contain more energy than the original CO_2 and H_2O. Consequently, photosynthesis can be regarded as a process of converting the radiant energy of the sun into chemical energy of plant tissues.

Photosynthesis occupies a central place in the metabolism of algae as it creates a foundation for the consideration of their other chemical activities. The primary products of photosynthesis are the high-energy molecules of adenosine triphosphate (ATP), and the hydrogen donor nicotinamide adenine dinucleotide phosphate ($NADPH_2$). These products are known to be involved in a wide variety of biochemical reactions within algal cells: therefore photosynthesis is directly intermeshed with and influences almost all of the life processes in the cell. Furthermore, the product of the photosynthetic reaction of carbon dioxide is not immutable and may be not only glucose, which has classically been regarded as the specific product, but also any of a

variety of other organic substances according to the physiological state of the cell (for example, starch).

In the photosynthetic process it is possible to distinguish between a series of reactions that are entirely dependent on light energy and involve the conversion of radiant into chemical energy ('light reactions') and a further series of reactions where a multiplicity of compounds are formed and interconverted to produce a fairly stable array of carbohydrates and other organic compounds ('dark reactions'). The light phase involves biochemical reactions with durations of 10^{-2} to 10^{-5} s. The biochemical events of the light phase result in 1) the production of the strong reducing agent $NADPH_2$, and 2) the formation of ATP, which is coupled to the flow of electrons and photons from H_2O to NADP. In the dark phase, the fixation of CO_2 occurs using the 'assimilatory power' of $NADPH_2$ and ATP (Figure 2.1).

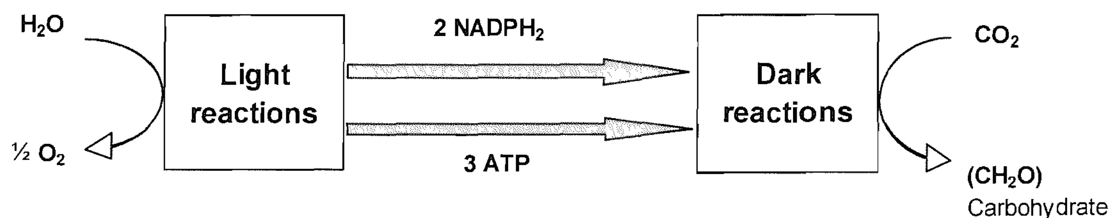


Figure 2.1 Major products of the light and dark reactions of photosynthesis (from (Hall & Rao 1999)).

The immediate extent of photosynthesis performed by an alga depends directly on the quantity of light and the presence of three main elements namely hydrogen, oxygen and carbon. While hydrogen and oxygen are derived from water molecules and for algal photosynthesis both elements exist in abundance in the medium itself, the concentration of carbon and available light intensity are limiting factors for photosynthesis.

2.2.2 Carbon sources

Carbon is recognised to be the principal structural component of any living organism: this is based on the fact that any energy transformations in the metabolic processes of an organism occur by chemical changes in carbon compounds.

Input of carbon dioxide into an aqueous system can occur in different ways, one of which is diffusion of CO_2 at the air-water interface. Although wind mixing can enhance the transport of CO_2 from the air into the system, the diffusion coefficient of CO_2 in water is 10^{-5} times that in air. The average concentration of CO_2 in dry air is only 0.035%, which may not be enough to sustain optimal growth and productivity of an algal population (Becker 1994; Nazaroff & Alvarez-Cohen 2001). Furthermore, CO_2 transfer from air can only occur on the air/water.

While terrestrial plants are mainly exposed to the gaseous form of carbon dioxide, aquatic plants including algae are open to three forms of inorganic carbon:

- CO_2, dissolved carbon dioxide,
- HCO_3^-, bicarbonate, and
- CO_3^{2-}, carbonate ions.

The actual concentrations of these forms will depend on several factors mainly the concentration of hydrogen ion in the surrounding medium, the partial pressure of carbon dioxide in the atmosphere, and temperature.

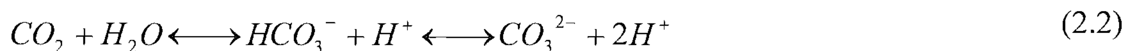
Fogg (1972) analysed the available data and concluded that, in general, the total concentration of all forms of carbon dioxide in water is sufficient or at least not limiting for photosynthesis. In contradiction to this conclusion, some researchers consider dissolved inorganic carbon in form of CO_2 as an important and potentially limiting substrate for algal photosynthesis (King 1970; King & Novak 1974; Novak & Brune 1985). Experimental evidence has confirmed however that many algal species are able to photosynthesise at substantial rates at a pH as high as 11, at which the concentration of free CO_2 is negligible (Tsuzuki *et al.* 1985).

In an early work, Osterlind (1949) found a lag period in HCO_3^- utilisation by *Scenedesmus quadricauda* and ascribed it to an activation period of either bicarbonate

absorption or carbonic anhydrase formation. Thielmann et al (1989) presented evidence indicating that *Scenedesmus* is able to utilise both undissociated carbon dioxide and bicarbonate ions; only 10 to 20 μmol l^{-1} of CO_2 being required for maximum growth whereas the corresponding value for undissociated carbon dioxide is 80 μmol l^{-1}. On the other hand, similar experiments with *Chlorella pyrenoidosa* (Fogg 1953) and *Chlorella vulgaris* (Tsuzuki *et al.* 1985) show that the species are not able to utilise bicarbonate, which may be explained by the structural impermeability of the cytoplasmic membranes to HCO_3^- ions. Thus there may be two types of algae with respect to carbon dioxide absorption.

It is known that un-ionised molecules penetrate cell membranes more rapidly than ions. Diffusion of CO_2 into the cell is therefore likely to be relatively unrestricted. Beardall (1985), Surif and Raven (1989) also suggested the involvement of active transport of inorganic carbon, accounting for the accumulation of CO_2 in the cells. Active absorption of anions is a property common to all growing plant cells, and there seems no reason to suppose that bicarbonate ions are exceptional in not being absorbed by this mechanism.

Carbonic anhydrase (CA) is a metalloenzyme (Zn^{2+}) that catalyses the interconversion of CO_2 and HCO_3^- (Khalifah 1971). The enzyme also participates in the transport of inorganic carbon to actively photosynthesising cells or away from actively respiring cells (Henry 1996). The presumed mechanism is that the enzyme generates a Zn-bound HCO_3^- by attacking a CO_2 molecule; the bicarbonate bound to the zinc is then replaced by a water molecule, liberating HCO_3^-. HCO_3^- in solution can gain a H^+ to form H_2CO_3 or can lose an additional H+ to form CO_3^{2-}. The overall relationship between three forms of dissolved inorganic carbon is shown in the equation:



$$CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+ \longleftrightarrow CO_3^{2-} + 2H^+ \qquad (2.2)$$

The uncatalysed hydration-dehydration reactions are slow, while the dissociation reactions are considered instantaneous (Moroney *et al.* 2001). CA significantly increases the hydration of dissolved CO_2, thus increasing the rate of equilibration of forms of inorganic carbon in solution.

One form of CA has been found extracellularly in green microalgae such as *Chlamydomonas reinhardtii* (Hewett-Emmett & Tashian 1996), *Dunaliella salina* (Fisher *et al.* 1996) and *Chlorella sorokiniana* (Satoh *et al.* 1998). This may indicate that an extracellular localisation could be common in aquatic photosynthetic organisms.

The equilibrium between forms of inorganic carbon is pH-dependent. At normal intracellular ionic strength, when the pH level is below the first dissociation constant (pK_1 = 6.4) CO_2 predominates; at pH between 6.4 and about 10.3 (pK_2) HCO_3^- predominates; while above pH of 10.3, CO_3^{2-} predominates.

Carbonate ions evidently cannot serve directly as a source of carbon dioxide and may have an inhibitory effect upon growth. Clear evidence of direct utilisation of bicarbonate ions is difficult to obtain since the ratio $[CO_2]$ / $[HCO_3^-]$ in solutions cannot be altered without also changing the hydrogen ion concentration. Furthermore, some algal species are able to utilise not only HCO_3^- but also CO_3^{2-}, but this is difficult to distinguish as CO_3^{2-} and HCO_3^- rapidly equilibrate with each other even at high pH (Golterman 1975).

The relationship between the rate of photosynthesis and carbon dioxide concentration depends strongly on various factors. For example, an increase in temperature in bright light leads to an increased rate of photosynthesis and consequently to higher uptake of CO_2. "The effect on the rate of a function of change in one factor depends on the level of the other factors to which the plant is exposed": this is the so-called law of limiting factors proposed by Blackman (1905) (as cited by (Kilham *et al.* 1979; Talling 1979; Cullen 1991). In other words, when a process is affected by several factors the rate of the process is determined by the factor that is in shortest supply. Complications arise due to the fact that plants are able to a certain extent to adapt to changing environmental conditions. For example, Ramazanov and Semenenko (1988) showed the similarity of the Photosynthesis-Irradiance curve and CA activity curve in response to changing irradiance for *Chlorella* species. This suggests that light intensity plays a modulating role in CA activity in *Chlorella*. High levels of irradiance stimulate photosynthetic carbon uptake, thus reducing free CO_2 in the solution and increasing CA activity as a response to the carbon demand.

Carbon has often been suspected of being a limiting factor because of the high algal demand for it, while its concentration in highly eutrophic waters may be relatively low compared to other nutrients (Goldman & Toerien 1972). In this connection, the retention time and BOD organic loading might provide the necessary regulation mechanism through bacterial respiration to algal demand (Azov *et al.* 1982). It must be remembered that the rates of the principal chemical reactions that involve the solubility of carbon in water are quite different. Whereas the ionisation reactions

$$CO_3^{2-} + H^+ \longleftrightarrow HCO_3^- \qquad (2.3)$$

and

$$HCO^- + H^+ \longleftrightarrow H_2CO_3 \qquad (2.4)$$

are almost instantaneous, the dissociation of carbonic acid is a relatively slow process with k (H_2CO_3) = 26.6 s^{-1}:

$$H_2CO_3 \longleftrightarrow H_2O + CO_2 \qquad (2.5)$$

At low concentrations the rate of photosynthesis is found to be proportional to carbon concentration, whereas at high concentrations saturation is approached and the rate becomes independent of concentration.

The limitation of carbon uptake by light supply has been demonstrated in microalgae (Beardall 1985), where the inorganic carbon dose-response curves were suppressed when measured at low photon flux density (PFD) compared to saturating values. The relationship between phytoplankton physiology and nutrients is usually described by two related models. The first model is based on the Michaelis-Menten equation (Bitton 1998), which describes the relationship between nutrient uptake and ambient limiting factors such as nutrient concentration, light, and temperature.

$$V = \frac{V_{\max}[S]}{K_m + [S]} \qquad (2.6)$$

where V is the reaction rate (unit/time), V_{max} is the maximum reaction rate (units/time), $[S]$ is the substrate concentration (mol l^{-1}) and K_m is the half saturation

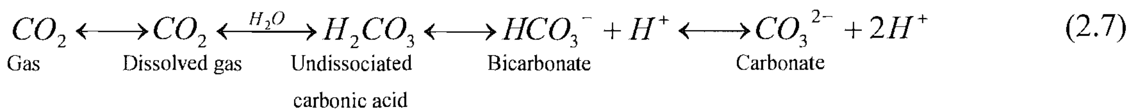
constant (Michaelis constant; the substrate concentration at which V is equal to $V_{max}/2$).

The second model is the Monod model, which expresses the relationship between growth rates and ambient limiting factors such as any of those enumerated above. This will be discussed in section 2.5 ("Algal growth in batch culture").

2.2.3 pH shift

During active algal photosynthesis, the pH in high rate ponds is often observed to rise as high as 11 in the afternoons during the warm season (Nurdogan & Oswald 1995). The change in hydrogen-ion concentration influences the chemical state of many algal nutrients such as phosphate, ammonia, iron and trace metals, and carbon dioxide.

Carbon dioxide dissolves in water to form soluble carbon dioxide that reacts with water to produce undissociated carbonic acid (H_2CO_3), which in turn dissociates and equilibrates as bicarbonate (HCO^{3-}) and carbonate (CO_2^{3-}). Equation 2.7 shows a series of reversible chemical changes that control pH in water reservoirs.



$$CO_2 \longleftrightarrow CO_2 \overset{H_2O}{\longleftrightarrow} H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \longleftrightarrow CO_3^{2-} + 2H^+ \qquad (2.7)$$

During the photosynthetic process algae will deplete in the first place free CO_2 and secondly HCO_3^-, thus altering the equilibrium towards a decrease in H^+ concentration. However, the pH shift is reduced by the existing reservoirs of carbonate and bicarbonate present in a waterbody. The inorganic carbon equilibrium is the major pH buffering system for the waterbodies, and generally remains at a pH between 6 and 9.

However, in a poorly buffered system, as it is a case with dense algal suspensions, the assimilation of carbon dioxide or bicarbonate by the rapidly growing algae cases a shift of the above equilibrium resulting in elevated pH values due to excretion of OH^- ions by the algae into the medium.

The relative concentrations of inorganic carbon species determine the pH of the effluent and in turn are determined by the pH (Figure 2.2). At night algae continue to respire and produce CO_2, which lowers the pH and causes daily pH fluctuations.

Goldman *et al* (1981) suggested that algal limits in pH tolerance mostly depend on the toxicity of the carbon species to the algal cells or the chemical alteration of the medium caused. The influence of inorganic carbon availability to the algae was not included in the factors affecting pH tolerance, however.

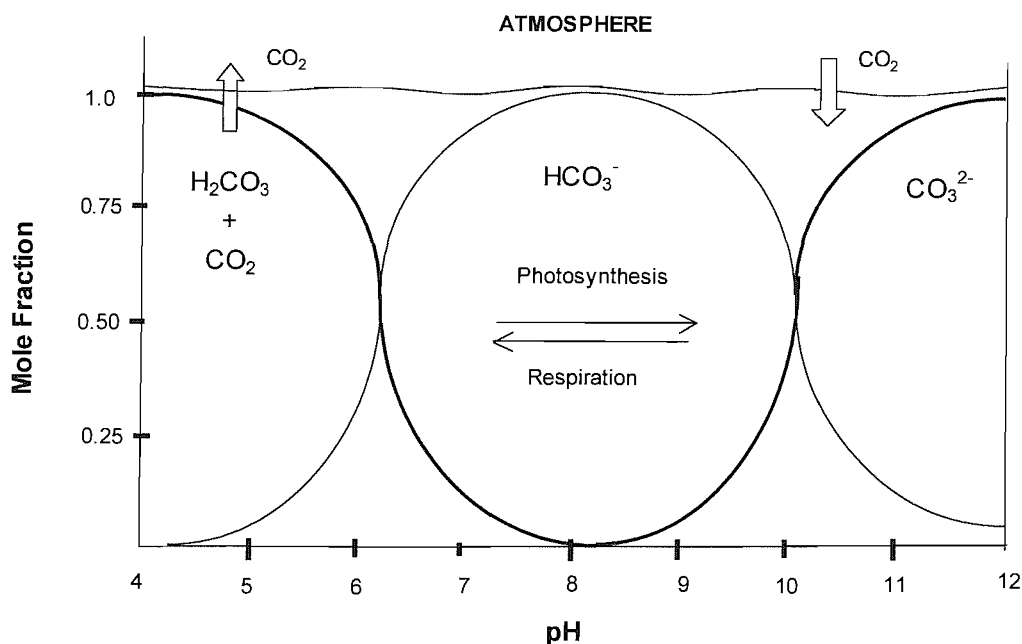


Figure 2.2 Effects of photosynthesis and respiration on pH, the ionic forms of inorganic carbon in the solution, and CO_2 exchange at the air-water interface (from (Richmond 1986)).

2.2.4 Light and photosynthesis relationship

The relationship between light intensity (*I*) and photosynthesis (*P*) is frequently presented as the light-saturation or *PI* curve (Figure 2.3). The *PI* curve can be divided into two phases. At low light levels, the rate of photosynthesis increases linearly as the irradiance level is increased. The initial slope of the curve at low light, α, is a function of both light-harvesting efficiency and photosynthetic energy conversion efficiency, and thus is a measure of the photosynthetic efficiency with which algal cells utilise light energy.

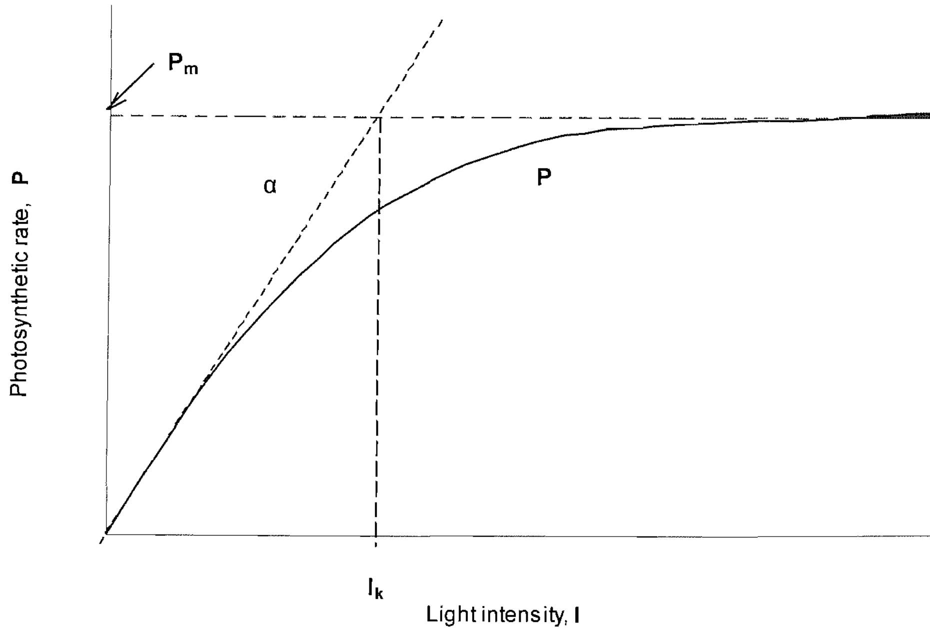


Figure 2.3 Schematic representation of specific photosynthetic rate (P) as a function of Light Intensity (I) (adapted from Kirk 1994)).

Photosynthetic efficiency is a measure of the capacity of the light-dependent reactions; cells that can efficiently absorb light and pass that energy to ATP and NADH will have higher photosynthetic efficiencies (steeper slopes) in the *PI* curves.

The rate of photosynthesis eventually levels off, after reaching the 'light saturation point'. The value of the light saturation point is a measure of the capacity of the light-independent reactions, and indicates the maximum rate at which the cells can fix CO_2 into carbohydrates. Above this value, the light provides more energy than the light-independent reactions can use – the process is 'saturated'.

Up to the establishment of saturation, the photosynthesis versus light intensity parameters can be calculated from following equation (Jassby & Platt 1976):

$$P = P_{\max} \tan(\frac{\alpha \times I}{P_{\max}}) \tag{2.8}$$

where P is the specific photosynthetic rate, P_{max} is the maximum specific photosynthetic rate, I is the growth light intensity (μmol $m^{-2}s^{-1}$), α is the slope of the linear part of the *PI* curve and is regarded as the light harvesting efficiency.

2.2.5 Implications for WSP systems

The presence of significant concentrations of oxygen is an important factor supporting microbiological activity in the aerobic zone of a facultative pond. Algal photosynthetic oxygen production is controlled by number of limiting factors of which the more important ones are light (as light intensity and light availability), temperature, nutrients (such as nitrogen, phosphorus and especially inorganic carbon), and pH.

As demonstrated by Oswald (1963) algal systems have the versatility of not only providing oxygen for BOD removal, but also incorporating nutrients such as nitrogen and phosphorus into cell tissue, and thereby reducing the eutrophication potential in receiving waters. The major drawbacks of WSP systems are that large areas of land are required, the process is effective only in areas of high sunlight intensity and, to avoid subsequent release of nutrients in receiving waters, physical separation of the microalgae may be required (Beneman *et al.* 1977).

Field experience with WSPs has shown that the pH of the pond effluent can experience transient changes during daytime (at initial pH around 8.0 in the morning to 11.0 at midday). This change is dictated by the extensive uptake of inorganic carbon species by algal pond population as discussed above. It may be interesting to compare rates of carbon uptake in batch systems with controlled and uncontrolled pH, as theoretical considerations indicate that the rate of carbon fixation is not a function of bulk inorganic carbon concentration or bulk CO_2 concentration alone, but is also dependent on the bulk pH because of its interaction with inorganic carbon.

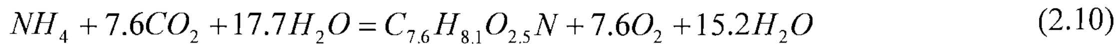
2.2.6 Algal Nutrient Composition

Based on the photosynthetic equation and considering other essential nutrients, particularly nitrogen and phosphorus, it is possible to write an overall equation for synthesis of algal biomass (Bush *et al.* 1963):

$$CO_2 + NO_3^- + PO_4^{3-} + H_2O \xrightarrow{h\lambda} [CH_2ONP]_n + O_2 \qquad (2.9)$$

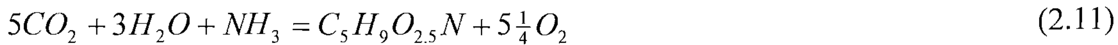
More than 20 trace elements that are required for growth of algal species are normally present in wastewater in variable amounts (Oswald 1988). As already stated, the synthesis of cell material by the growing algae is a mechanism for the removal of many dissolved solids from wastewater.

According to Oswald (Oswald 1963) the overall cell synthesis equation for green algae growing on wastewater is:



$$NH_4 + 7.6CO_2 + 17.7H_2O = C_{7.6}H_{8.1}O_{2.5}N + 7.6O_2 + 15.2H_2O \qquad (2.10)$$

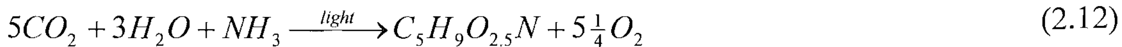
Alternatively McKinney(1982) as cited by (Prince *et al.* 1994) suggested:



$$5CO_2 + 3H_2O + NH_3 = C_5H_9O_{2.5}N + 5\tfrac{1}{4}O_2 \qquad (2.11)$$

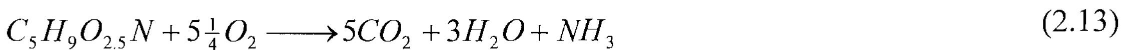
Algae use energy from light to combine stable CO_2 with water, nitrogen, phosphorus and other elements to produce unstable protoplasm and oxygen. This cell protoplasm undergoes continual endogenous respiration that appears as the reverse of the first reaction.

Using equation 2.11, algal photosynthesis is represented by



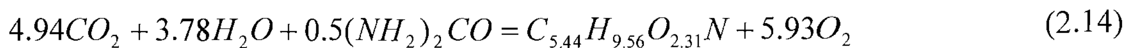
$$5CO_2 + 3H_2O + NH_3 \xrightarrow{light} C_5H_9O_{2.5}N + 5\tfrac{1}{4}O_2 \qquad (2.12)$$

Algal degradation then is:



$$C_5H_9O_{2.5}N + 5\tfrac{1}{4}O_2 \longrightarrow 5CO_2 + 3H_2O + NH_3 \qquad (2.13)$$

Doney and Myers (1958) found a thermophilic strain of *Chlorella pyrenoidosa* had an average composition of $C_{5.44}H_{9.56}O_{2.31}N$ when grown continuously in bright light with urea as the nitrogen source. The synoptic equation for the complete process should thus be written:



$$4.94CO_2 + 3.78H_2O + 0.5(NH_2)_2CO = C_{5.44}H_{9.56}O_{2.31}N + 5.93O_2 \qquad (2.14)$$

The composition of algae varies somewhat depending on age because of this endogenous respiration. Young algal cell composition is of the form $C_5H_9O_{2.5}N$, while

older algae cells have a composition of $C_9H_{17}O_6N$ (McKinney 1982) as cited by (Prince *et al.* 1994).

When nutrients are provided in excess and light is the growth-limiting factor, most algal species display a remarkable consistency in their chemical composition of approximately 45-50% carbon, 8-10 % nitrogen, 1% phosphorus (Spoehr & Milner 1949; Myers 1964; Goldman 1979).

2.3 Removal of Nutrients: Nitrogen and Phosphorus

It is clear from the above that the synthesis of cell material by growing algae is a mechanism for the removal of many dissolved inorganic chemical species from wastewaters. The final effluent from a conventional treatment plant, however, is rich in inorganic nitrogen and phosphorus which, under suitable environmental conditions in the receiving watercourse, can cause eutrophication. Taking into account different sources of wastewater, there are also other potential problems resulting from discharge of refractory organics and heavy metals, as well as residual pathogenic microorganisms (Noüe *et al.* 1992). Compared to natural water bodies the concentrations of nitrogen and phosphorus found in effluents of different origin may be up to several orders of magnitude higher (Table 2.1).

Nitrogen is reported as one of the components of wastewater that is typically difficult to reduce to levels that do not cause the eutrophication in the receiving waters (Horne 1995). Algae provide an effective way of removing nitrogen from wastewater effluent by means of accumulation of the nutrient inside cells and its use in growth. Green algae are important because they can assimilate NH_4^+, NO_2^- and NO_3^- at a considerable rate, interchanging sources of inorganic nitrogen if necessary.

Several factors will affect the maximum rate of inorganic nitrogen uptake, namely presence of substantial concentration of carbon source, saturating light intensities, and optimal temperature regime. Light and temperature effects upon nitrogen uptake at saturating concentrations of other nutrients are still poorly understood and require further investigation.

Table 2.1 Range of total nitrogen and phosphorus concentrations found in various effluents (after (Noüe *et al.* 1992)).

Type of effluent	Total nitrogen concentration (mg l^{-1})	
	Nitrogen	Phosphorus
Lake water	0.2 – 0.5	0.005 – 0.261
Fish farm wastewater	Approximately 3	0.1-0.3
Urban wastewater	Approximately 5	3-15
Tomato concentrate production	15 –70	-
Rubber production	164 – 295	45-86
Pig waste	300 - 4000	50-1500

2.3.1 Methods of nitrogen removal in WSPs

WSP influent commonly contains large amounts of nitrogen (N) relative to that found in lakes, streams, and oceans. Concentrations of nitrogen in the form of ammonia higher than 1 mg N l^{-1} at pH values over 8 are potentially toxic to wildlife in most natural water reservoirs. Concentrations of nitrogen in the form of nitrate at around 1 mg N l^{-1} are sufficient to cause eutrophication in receiving streams. An advantage of WSPs is that around 50% of nitrogen is normally present in form of particulate-N such as algae and bacteria (Senzia *et al.* 2002), giving the potential for removal by sedimentation, precipitation or filtration.

Middlebrooks *et al.* (1982) suggested three major mechanisms responsible for nitrogen removal from WSPs:

- volatilisation of nitrogen (in the form of ammonia) at a pH above 8.0;
- biological nitrification coupled to denitrification, and
- assimilation of nitrogen into algal biomass.

The role of sedimentation processes has been also recognised ((Ferrara & Avci 1982; Muttamara & Puetpaiboon 1996). The relative importance of any of these mechanisms clearly depends on the design, operational approach and type of the pond (Azov & Tregubova 1995; Gomez *et al.* 1995; Silva et al. 1995; Soares *et al.* 1996).

Ammonia nitrogen can exist in an aqueous solution as either NH_4^+ ions or NH_3 gas. The pH and temperature of the medium affects the balance according to the following equilibrium reaction:

$$NH_{3(gas)} + H_2O = NH_4^+ + OH^- \quad (2.15)$$

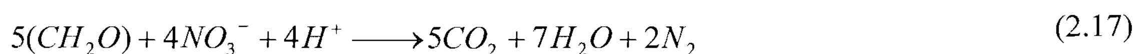
The equilibrium is shifted to the left in favour of NH_3 gas at pH values above neutral, while the NH_4^+ ion is the predominant species at pH values below 8.0 (Nazaroff & Alvarez-Cohen 2001). Soluble ammonia can be removed by volatilisation directly to the atmosphere as ammonia gas.

In WSPs the amount of nitrogen removed by volatilisation depends on the retention time, surface area of the pond and pH fluctuations (Middlebrooks *et al.* 1982). Some disagreement is encountered in the literature regarding the relative importance of the volatilisation process versus biomass uptake of nitrogen compounds, however. Curtis and Mara (1994) pointed out that the use of the ammonia volatilisation on its own is not a practical consideration in achieving a high percentage of nitrogen removal.

The removal of nitrogen by biological nitrification/denitrification is a two-step process. In the nitrification step, ammonia is converted aerobically to nitrate nitrogen (NO_3^--N):

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \quad (2.16)$$

In the denitrification step, nitrates are converted to nitrogen gas by denitrifying bacteria under anaerobic conditions:



$$5(CH_2O) + 4NO_3^- + 4H^+ \longrightarrow 5CO_2 + 7H_2O + 2N_2 \quad (2.17)$$

As a condition of compete denitrification a carbon/nitrogen ratio of at least 2:1 (based on total organic carbon (TOC) and total N) is required in natural wastewater treatment systems (Tchobanoglous & Burton 1991).

Azov and Tregubova (1995) reported that nitrification did not occur in Maale Kishon stabilisation reservoir, in Israel. Ferrara and Avci (1982) have assumed that nitrification does not normally occur in stabilisation ponds based on low concentrations of nitrite and nitrate found in the pond effluent. However, results of Zimmo *et* (2002) showed that nitrification as well as denitrification are taking place in both algae based and macrophytes based wastewater treatment systems. Denitrification is suggested as taking place at the mud/water interface even though the water is never anoxic (Ellis 1983). According to Reed (1985), denitrification is only a theoretical possibility for permanent nitrogen loss in ponds and nitrification-denitrification is not likely to be a major mechanism for nitrogen removal in a pond system.

The influence of phytoplankton biomass on the amount of inorganic nitrogen present in the effluent is due to two reasons. Firstly, the high values of pH observed in WSP are mostly due to active algal uptake of carbon during the photosynthetic process. The high pH values of the effluent would stimulate ammonia volatilisation. Secondly, the uptake of nitrogen by phytoplankton during active growth accounts for up to 10 % of algal biomass weight. Approximately 50% of nitrogen in WSP effluent is found to be present in form of algal and bacterial biomass (Senzia *et al.* 2002).

2.3.2 Nitrogen sources for algal growth

Protein synthesis requires nitrogen in addition to oxygen, hydrogen, and carbon. After carbon, nitrogen is quantitatively the most important single element contributing to the dry matter in algal cells. In an exponentially growing culture of microalgae, nitrogen is reported to be about 7-10% of the dry matter (Richmond 1986). Actively growing wastewater-borne algae have been reported to contain around 8% of nitrogen in their dry weight (Green *et al.* 1996), and therefore secure a proportional removal of nitrogen from WSP effluent with biomass removal.

There are several forms of nitrogen-containing compounds such as nitrate, nitrite, ammonia and organic sources of nitrogen such as urea, which are available as a source of nitrogen for green algae in WSP.

There appears to be no clear relationship between the ability to use a particular group of nitrogen compounds and the taxonomic class of algae (Antia *et al.* 1975). Almost all chlorophyll-containing algae studied in culture will grow with nitrate (NO_3), nitrite (NO_2) or ammonium (NH_4) as a nitrogen source (Becker 1994). Kaplan *et al.* (1986) reported that maximum growth rates are generally similar with either NO_3 or NH_4 as an N source. However, preference is given to ammonia (Harvey 1953). Note that 'ammonia' is used as a general to denote the substrate, whether the form in which it is taken into the cell and subsequently metabolised is ammonia (NH_3) or ammonium ion (NH_4^+).

Inside the cell ammonia is converted to organic nitrogen compounds and its assimilation takes place at the expense of endogenous carbohydrate reserves (Fogg 1953). The pathway for ammonia utilisation has been described for green algae, where glutamic acid is the first compound (Becker 1994). On the other hand, ammonia concentrations exceeding 2 mM, especially in combination with pH values higher than 8, have been reported to be toxic for phytoplankton (Abeliovich & Azov 1976). Such concentrations are not uncommon for various types (domestic, industrial, or agricultural) of wastewater.

At high pH values nitrate becomes the most important source of inorganic nitrogen. Two enzymes systems, namely nitrate reductase and nitrite reductase, catalyse the entire reduction of nitrate to ammonia in microalgae:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NH_4^+ \qquad (2.18)$$

As is clear from equation (2.18), nitrite can be also used as source of inorganic nitrogen. However, nitrite is highly toxic for microalgae in concentrations exceeding 1 mM (Morris 1974).

As demonstrated by Eppley (1972), the growth rates of pure cultures on nitrate and ammonium are found to be comparable at similar growth conditions. There is a lack of firm evidence that the growth of algal cells on nitrate is disadvantaged in any way

compared to growth on ammonium, although the theoretical cost of nitrate reduction to ammonium is 25% of the photosynthetic energy gain (at a light intensity of 200 μmol m^{-2} s^{-1}) as calculated on thermodynamic grounds by Guerrero *et al.* (1981).

There are four potential strategies which algae utilising nitrate may use to compensate for the higher energy requirement of nitrate reduction. In order given by Levasseur *et al.* (1993), there are:

1) a decrease in growth rate and/or in carbon and nitrogen quotas;

2) an increase in light absorption efficiency or through in pigment content;

3) an increase in net photosynthetic efficiency;

4) use of different, more energy-efficient, metabolic pathways.

However, many studies have failed to reveal any differences between growth of cells on ammonium and nitrate. Paasche (1971), Rhee and Lederman (1983) have provided some evidence that a number of phytoplankton algae grow more rapidly on nitrate (although this is perhaps due to the toxicity of ammonium when supplied in high concentrations). Any difference in growth rates between nitrate- and ammonium-growing cells may also be influenced by light intensity, as supported by experimental evidence presented by Thompson *et al.* (1989).

Since ammonia is the end product of nitrate reduction, it causes feedback inhibition and the repression of nitrate uptake (Becker 1994). It was found that growth rates in urea are generally similar to those in NO_3 and NH_4 (Syrett 1988).

2.3.3 Algal uptake of nitrogen

Since nitrogen is found to be a growth-limiting nutrient for many natural aquatic environments, a considerable volume of literature exists in connection to assimilation and uptake kinetics of the nutrient. In WSPs on the other hand, the nutrient is reported in abundance due to the nature of typical wastewaters. Valuable experience in studying nitrogen uptake in nitrogen-limited environments can however be applied to the specific conditions in WSPs.

Numerical values for the maximum uptake rate, V_{max}, which is a key parameter in the Michaelis-Menten model (equation 2.6), vary among planktonic algae. The differences in maximum uptake rates have been used to explain mechanisms that lead to changes in community structures, when supply modes of limiting nutrients change.

Uptake rates of inorganic nitrogen and the rate of growth in a nitrogen-limited environment of phytoplankton can be adequately modelled by a hyperbolic relationship such as the Michaelis-Menten (2.6) or Monod equations (2.28) (Legovic & Cruzado 1997). In waters with a considerable level of eutrophication values of the Michaelis-Menten K_m and V_{max} were observed to be higher than 1 μM and 0.01 h^{-1} respectively (Collos & Slawyk 1980). Brown and Johnson (1977) quoted values of the Monod K_s for nitrate equal to 32 $\times 10^{-6}$ M for natural freshwater populations and 50×10^{-6} M for *Chlorella pyrenoidosa.* Carpenter and Guillard (1971) showed that clones of the same species had different K_s values depending upon their environment. Thus clones isolated from nitrogen-poor waters had K_s values of $< 0.75\times10^{-6}$ M, while the same species taken from nitrogen-rich region has K_s of $< 1.5\times10^{-6}$ M. Such findings are significant as they show that no generalisation of K_s values is possible for different systems (for example, between batch and continuous cultures). It was shown by Eppley at al. (1969) that the K_s value for nitrogen could range between 0.1 - 10 μM with the lower values characteristic of species from an environment with low concentrations of the nutrient.

The ratio V_{max} / K_m, often defined as the affinity (A), incorporates both parameters and is the initial slope of the uptake rate versus substrate concentration curve. A provides an index describing the ability of cells to accumulate substrate, while the parameter is independent of the uptake mechanism (Button 1985; Button 1986). However, deviations from the Michaelis-Menten equation such as linear uptake (MacIsaac & Dugdale 1969) with concentration and inhibition phenomena (Harrison *et al.* 1977) have been also reported. Limitations in the application of the equation were considered in cases where specific uptake rate was influenced by difference in cell nitrogen quota (Dugdale 1977).

A common feature for the uptake system of various species is the substrate is accumulated within the cells sometimes reaching a concentration 10^2 or 10^3 higher

than the external concentration, so-called 'luxury consumption' (Syrett 1981). The cell pool of nitrogen is divided into two groups inside the unit: functional substrates (such as proteins and free amino acids), which are involved in growth processes, and storage substances that are not available for growth (insoluble N in cell walls, membrane proteins and nucleic acids) (Naldi & Wheeler 1999) (Figure 2.4).

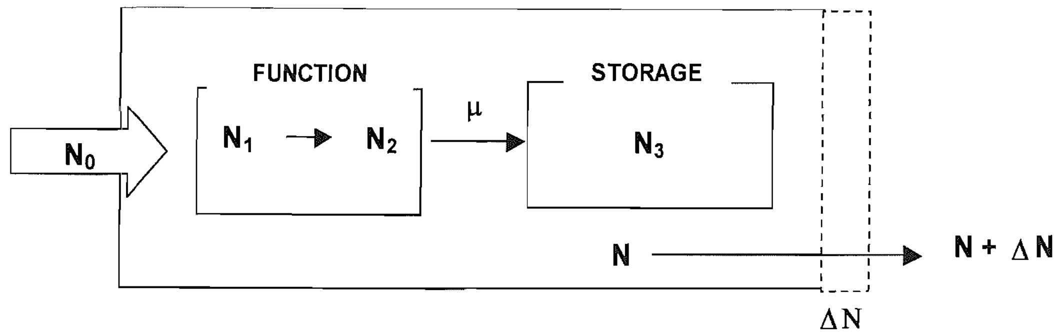


Figure 2.4 Summary of nitrogen flow into the cell (adapted from (Collos & Slawyk 1980)).

According to Eppley (1969) V_{max} is the best taken as the maximum specific growth rate of the organism imposed by external conditions, rather than from measured uptake rates uncoupled from growth, and thus is a variable and subject to the effects of irradiance and temperature as well as species-specific properties of the organism.

2.3.3.1 Importance of light and carbon availability to nitrogen uptake

N-sufficient green algae do not take up and reduce NO_3^- in the absence of CO_2 (Syrett 1981). Van Niel *et al.* (1953) observed the effect of light stimulation on nitrate uptake by *Chlorella* species. At a saturation light intensity, the rate of oxygen evolution by *Chlorella* was increased by addition of NO_3 while CO_2 assimilation was unchanged. Studies with natural marine phytoplankton suggest that light influences inorganic nitrogen uptake. Thacker and Syrett (1972) demonstrated that *Chlamydomonas* as well as *Chlorella* did not assimilate nitrate or ammonia unless a suitable source of carbon was provided.

Charged ions such as NH_4^+ and NO_3^- are taken up by means of carrier-mediated mechanisms. Both active and passive carrier-mediated transport mechanisms depend

on a finite number of saturable carrier (enzyme) sites. The photosynthetic process provides an ultimate source of energy in form of ATP and reducing power for nitrogen assimilation into protein. This can occur directly, either through the light-dependent production of ATP (Smith *et al.* 1987; Weger & Turpin 1989) and reduced ferredoxin, or indirectly through the respiration of previously fixed carbon compounds such as starch (Weger & Turpin 1989).

It has also been reported that the relationship between light intensity and rate of nitrogen uptake in unicellular algae appears to be a rectangular hyperbolic function that can be adequately modelled by the Michaelis-Menten equation (MacIsaac & Dugdale 1969). Half-saturation constants for light-related nitrate uptake generally are very low, for example, light intensity ranging from 0.9 to 14% (0.002-0.057 ly min^{-1}) for nitrate and from 1.3 to 4% (up to 0.008 ly min^{-1}) for ammonium uptake (MacIsaac & Dugdale 1972). Studies with natural communities have shown that the light dependence of inorganic nitrogen uptake has species-specific properties (Conway & Whitledge 1979).

2.3.3.2 Temperature effects on nitrogen uptake

Relatively little is known about the temperature sensitivity of inorganic nitrogen uptake by microalgae (Reay *et al.* 1999). Physical-chemical models of uptake predict a positive relationship between the half-saturation constant for uptake and temperature(Aksnes & Egge 1991). In laboratory studies, the rate of nitrate uptake has been shown to be related to temperature in higher plants (Guy 1990) and in diatoms (Gao *et al.* 2000). A low affinity for inorganic nutrients in oceanic phytoplankton at suboptimal temperatures has also been reported (Gao *et al.* 2000).

2.3.4 Methods of phosphorus removal in WSPs

To prevent eutrophication in receiving streams the concentration of phosphate should not exceed 0.1 mg l^{-1}. (Surampalli *et al.* 1995) reported that in the US the influent phosphorus concentration varied from 0.5 to 5 mg l^{-1}. Mean wastewater phosphorus concentrations in France (Mesple *et al.* 1995) are recorded as 4.6 mg l^{-1} with a range

from 0.45 to 8 mg l^{-1}. These values may be taken as reflecting typical concentrations of phosphorus in wastewater. Thomann and Mueller (1986) found that the half-saturation constant of phosphates for algae was 2.5 μg l^{-1} and therefore the average concentration of phosphorus ($P\text{-}PO_4$) in WSP effluents tends to exceed acceptable discharge levels.

In municipal wastewater treatment there are two means by which phosphorus is removed: chemical precipitation (Simmonds 1973) and various biological treatment processes (Mulkerrins *et al.* 2004). In WSP treatment systems, phosphorus is also removed by environmentally sound means such as assimilation into the biomass of algal and bacterial population. The increase in pH due to algal carbon dioxide uptake also causes a decrease in bicarbonate alkalinity, leading to acceleration in precipitation of phosphates (Picot *et al.* 1991). It has been reported that as a result of these mechanisms WSP effluent contains less than 50% of the influent wastewater concentrations of phosphorus (Surampalli *et al.* 1995).

2.3.4.1 Sources of phosphorus for algal growth

Along with orthophosphate and pyrophosphates *Chlorella* species were found to be able to utilise organic sources of phosphorus such as urea phosphate and phospholipids (Galloway & Krauss 1963; Miyachi *et al.* 1965). *Scenedesmus* species on the other hand, have been shown not to use pyrophosphates or organic phosphorus sources (Fogg 1973). It has been suggested that the location of enzyme complexes is responsible for the choice of source of phosphorus. While in *Chlorella sp.* acid phosphatase is located on outside cell walls, for *Scenedesmus sp.* cells the enzyme was not found at such locations.

The main form in which algae acquire phosphorus is as inorganic phosphate, either as $H_2PO_4^-$ or HPO_4^{2-} (Becker 1994). It may, however, be a rather theoretical question as to whether algae can utilise a range of phosphorus sources or not. Nurdogan and Oswald (1995) reported that complete hydrolysis of polyphosphates and the decomposition of organic phosphorus compounds occurs under the influence of bacterial phosphatase activity in HRP. As a result, around 80% of total phosphorus is present in form of orthophosphate.

2.3.4.2 Algal uptake of phosphorus

Algae are a good tool for phosphorus removal in WSPs as phosphorus is in constant demand as one of the key nutrients required for normal growth of the organisms. It plays a major role in most cellular processes, particularly those involved in energy transfer and nucleic acid synthesis (Richmond 1986). The optimum phosphorus concentration in the medium, as well as the phosphorus tolerance, varies with different species, even if all other nutrients are supplied in sufficient concentrations. The average range for most algae is between 50 μg l^{-1} and 20 mg l^{-1}.

The uptake of orthophosphate is an active process for which energy may be supplied by photosynthesis (Fogg 1973; Kaplan *et al.* 1986) as well as by respiration and hence it is usually stimulated by light (Becker 1994). During photophosphorylation the photosynthetically produced energy is bound to the energy-rich phosphorus ATP that serves as a central carrier for all energy-requiring processes in the cell. In aerobic cell respiration, electrons are passed through an electron transport chain to form ATP in oxidative phosphorylation (or electron transport phosphorylation). Additionally, some amount of ATP is synthesized by a direct transfer of phosphate from a substrate molecule to ADP in substrate-level phosphorylation.

A commonly accepted phosphate interaction model is shown in Figure 2.5. In this model the transport of phosphorus (P) into the cell as PO_4^{3-} is subject to kinetic constraints from the external concentration, plus regulation via transinhibition from the amount of soluble organic phosphate. In reality it is most likely that P-transport will also be regulated by the concentration of internal organic-P compounds.

The phosphate (PO_4^{3-}) in the cells is channelled into various inorganic and organic phosphorus compounds. The general reaction for incorporation of orthophosphate into organic 'high energy' compounds is:

$$ADP + P_i \xrightarrow{energy} ATP \quad (2.19)$$

ATP has a central role in the metabolism of living organisms, since its hydrolysis provides the energy needed for most energy-requiring reactions such as CO_2 fixation, ion uptake and transport, formation of nucleic acids, and many others (Figure 2.5).

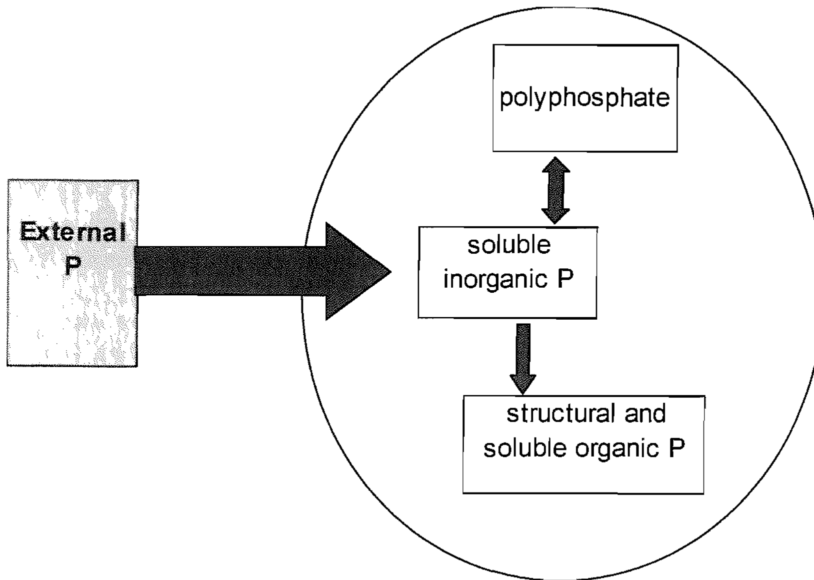


Figure 2.5 A schematic representation of transport in and distribution of phosphorus inside microalgal cells (modified from (John & Flynn 2000)).

Under sufficient phosphorus supply, inorganic phosphates are accumulated in algae as acid-labile polyphosphates granules, which are metabolised under phosphorus deficiency (Becker 1994). According to John and Flynn (2000) polyphosphates occupying less than 1% of cell volume could support a generation of cell growth with little or no P-stress.

Michaelis-Menten equation (2.6) describe the relationship between nutrient uptake and ambient nutrient concentrations, but its use for phosphorus is rather limited as relative growth rate is more dependent on intracellular concentration rather than the rate at which the nutrient enters the cell (McCarthy 1981). The difficulties in assessing the true phosphate uptake by phytoplankton are discussed in Lean and Nalewajko (1979). Phosphorus requirements for optimum growth also differ considerably from species to species (Lang & Brown 1981) as well as a growth phase of single species (Spijkerman & Coesel 1998) even if no other external factors are limiting.

2.3.4.3 Factors affecting phosphorus uptake

Fogg (1973) quoted an experiment by Simonis and Urbach (1963) where the uptake of phosphorus from the surrounding medium by algae was stimulated by light, but only at high concentrations of phosphate in the medium (2.1×10^{-5} M). Light stimulation for *Scenedesmus sp.* in the presence of sufficient concentrations of carbon dioxide was not pronounced (Kylin 1966), due to direct competition with the photosynthetic carbon fixation cycle for energy produced in photophosphorilation (see discussion above). The uptake rate is also influenced by the phosphate concentration in the medium, the pH, and, in several algal species, by the availability of Na^+, K^+, Mg^{+2} or other heavy metals in the medium (Smith & Kalff 1983).

2.4 Seasonal and climatic effects on algae in WSPs

2.4.1 Introduction

Climate is perhaps the most important factor to be taken into account in WSP systems, as it is practically uncontrollable in large-scale systems and yet is one of the primary determinants of technical feasibility and operational performance. In the classical design with the principal types of ponds used in warm regions such as Africa, Middle East and South America, the intense solar radiation and high temperature provide excellent conditions for continuous wastewater treatment. In moderate climatic conditions, for example in Mediterranean Europe, environmental conditions are still in favour of WSPs in continuous operation.

In cold climates, however, WSPs experience two distinct seasons: winter and ice free (Prince *et al.* 1994). In cold climates ice cover isolates the water surface from the mixing forces of the wind. Ice formation occurs at 0 °C at the surface, but the temperature in the rest of the water body remains between 0 and 4 °C. This is because water is at its maximum density at about 4 °C and thus the ice and the coldest water float near the surface. Therefore the winter season is usually characterised by the predominance of anaerobic conditions in the pond system. Anaerobic conditions and sedimentation cause a reduction in BOD concentration by 30-50 % during the ice-covered period in the ponds (Vinberg *et al.* 1966).

The ice-free season can be further subdivided into spring and summer sub-seasons. The latter is characterised by the establishment of steady state conditions in the pond, while the former could be characterised as non steady-state. The spring turnover breaks down the stability of water column and allows mixing to occur.

Processes controlling the development of phytoplankton blooms in natural aquatic bodies and their fate have received attention over a number of years. Several factors are recognised as important in controlling the onset of spring bloom such as mixing and grazing, but mainly light and temperature (Fogg 1975). Mixing processes occurring during this period remedy nutrient and light deficiencies. Such movements carry up algal cells from the pond bottom sediments to the surface of the water body, allowing them to begin active photosynthesis (Tett & Edwards 1984).

Initiation of the spring phytoplankton bloom, according to the Sverdrup (1953) model developed for ocean waters, results from a combination of factors that include constantly changing irradiance at the water surface, attenuation of light in the water column by particulate and dissolved organic matter, and thickness of the surface mixed layer, which determines the average irradiance to which phytoplankton are exposed (Kirk 1994).

In addition, the role of temperature in conjunction with light changes has been shown to determine the time of the bloom. Mei *et al.* (2002) demonstrated that spring phytoplankton blooms were sensitive to temperatures above $0^{o}C$, as well as to irradiance and lateral advection. The suggestion was made that fluctuations in wind field and atmospheric temperature can modify the timing and extent of ice formation as well as the strength of water column mixing and water temperature, which will modify the timing, duration and spatial pattern of the phytoplankton bloom.

Research also suggests that active grazing controls and even cause a cessation of spring algal bloom (Steel 1963).

Compared to the long duration and slow processes of winter, the beginning of the spring bloom occurs on an event scale and obviously selects for species with a fast growth rate. The lack of understanding of the influence of spring conditions on pond environment does not allow efficient operation of WSPs during spring.

2.4.2 Light

The principal sources of oxygen in naturally aerated WSPs are photosynthetic oxygenation and surface re-aeration. Oswald *et al.* (1957) demonstrated that surface re-aeration plays a minor role in supplying oxygen to aerobic and facultative microorganisms in the aerobic environment. The bulk of the oxygen requirement is provided by the algae, however, which can produce oxygen only in the presence of an adequate supply of light. This condition makes solar radiation one of the main driving forces for successful WSP performance. The production of oxygen by algae in the system depends on the duration and intensity of sunlight, which is also a factor in natural disinfection processes (Wilkinson 1986). The availability of light to non-motile algae will be determined by many factors including the depth of the pond and retention time of the wastewater.

2.4.3 Light availability for algae

When considering the availability of the light energy to photosynthesising algal cells the following factors must be taken into account.

Studies of light absorption by water and chlorophyll molecules indicate that only light of specific wavelengths is likely to be used for aquatic photosynthesis. This part of the visible range between 400 and 700 nm is called Photosynthetically Active Radiation (PAR). Note that at any references to light intensity apply to PAR in units of μmol photons m^{-2} s^{-1} (or μmol m^{-2} s^{-1}).

Variations in available light will also depend on geographical location, season and the changes in light intensity over the course of a day (Boney 1975). By considering the geographical location, Oswald and Gotaas (1955) summarised maximum-minimum monthly changes in light intensity.

Neglecting any absorption by the atmosphere the diurnal changes in irradiance *I*, can be described by following equation:

$$I = I_{\max} \frac{1}{2}(1 + \cos \frac{2\pi \times t}{\lambda}) \tag{2.20}$$

where I_{max} is the maximal irradiance, λ is the variable day length, *t* is the fraction of λ and the abscissa is shifted so that t= 0 at 12 h (thus t = - ½ λ at sunrise and t = ½ λ at sunset).

A number of factors make it difficult to give and exact mathematical representation if diurnal changes in of irradiance in practice: abrupt changes in irradiance level at the beginning and at the end of day, variation in atmospheric absorption due to varying concentration of cloud, gases and dust; the percentage of scattered or reflected light which depends primarily on local conditions. It is frequently easier and more precise to use empirical measurements obtained for each individual study, than to attempt to calculate the actual values of light availability at any point in time.

There is a gradual attenuation in light intensity with depth. Thus light availability to phytoplankton in a water body is classified by zonation (Goldman 1994) as follows:

- the photic or euphotic zone is defined as the upper layer of a water body which receives adequate light for photosynthesis to occur, in other words, it is a region of net photosynthetic oxygen production by phytoplankton. In practical terms this is usually taken as the depth at which light intensity is 1% of the surface irradiance;

- the aphotic zone is a light depleted region, where no significant photosynthesis can occur.

The depth of each zone differs considerably depending on the location, time of day, colour of water, and concentration of suspended matter including algae.

The intensity of light in the underwater environment decreases exponentially with depth, as described by Beer's Law:

$$\frac{I_1}{I_0} = e^{-b\Delta y} \tag{2.21}$$

where I_0 is the light intensity registered at the surface of a water body, I_1 is the light intensity registered at a given depth, Δy is the distance between the two observations, and b is an extinction or attenuation coefficient.

In the most favourable conditions algae are able to utilise up to 2.6% (1.5% average) of sunlight during summer months (Goldman 1979). During winter months the efficiency of light utilisation is higher, which may be explained by absence of photoinhibition periods.

2.4.4 Photoinhibition and photoacclimation

Photoinhibition is the inhibition of photosynthesis at high light intensities. There is abundant evidence that photoinhibition occurs in natural phytoplankton communities (Kirk 1994). Phytoplankton organisms have developed certain adaptations avoid or adjust to the damaging effect of high light intensities, however. For example, motile forms can adjust their depth in the water column to find optimal light conditions; even non-motile algae are able to change their position to find improved light conditions by controlling their cell density (Boney 1975). Excessive irradiance can inhibit the photosynthesis and induce production of photoprotective pigments (Ibelings *et al.* 1994). *Chlorella* and *Scenedesmus* are known to posses xanthophylls which play an important role in photoprotection of the cells from the potentially damaging effects of light (Masojídek *et al.* 1999).

Pahl-Wostl and Imboden (1990) reported that a time delay from 0.5 to 1.5 h was observed in the development of the full effects of photoinhibition on algal photosynthesis. Thus, short periods of exposure to inhibiting light intensities may not affect the total daily oxygen production by the algal community.

The majority of algal species are capable of some degree of photoacclimation (the response of algae to changes in photon flux density and spectral distribution). In the aquatic environment, changes in light intensity are inextricably linked to changes in spectral distribution (Kirk 1994). Generally, the process occurs on time scale shorter than or comparable to that of cell generation (Falkowski 1991). Morphologically, photoacclimation may be accompanied by changes in cell volume or in the number and density of thylakoid membranes (Post *et al.* 1985). On the cellular level there are changes in pigment and lipid content and composition (Perry *et al.* 1981). At a physiological level, there are changes in the minimum quantum requirement for

photosynthetic oxygen evolution (Thornley 1974; Kaplan *et al.* 1986), respiration (Falkowski 1984), and growth rate (Litchman 1998; Litchman 2000).

2.4.5 Temperature

Environmental temperature is a factor to which algae respond continuously. Algae experience temperature fluctuations of many different types, including diurnal changes, seasonal changes and long-term inter-annual variability associated with natural climatic cycles. Temperature sets an upper limit on the rate of photosynthesis and on the phytoplankton growth rate through its influence on the enzymatic activity of cells.

The species succession over the spring season in temperate waters has been described as temperature controlled (Fogg 1975), with the cold-tolerant species occurring in early spring and those favouring warmer conditions following in late spring and early summer.

2.4.6 Algal photosynthesis and temperature relationship

Algae grown for several generations at low temperatures normally exhibit changes in photosynthetic response in one of the following ways:

- increased values of the maximum photosynthetic rate (Sheridan & Ulik 1976; Davison 1991);
- increased resistance to the low-temperature induced photoinhibition (Falk *et al.* 1990);
- reduced sensitivity to changes in temperature (Davison 1991);
- lower optimum temperatures for photosynthesis (Sheridan & Ulik 1976; Li 1980);
- reduced tolerance to high temperatures (Davison 1991).

Temperature changes in the outdoor environment often occur over a shorter time-scale than those employed in many laboratory studies, and, in some cases, plants experience an oscillating temperature regime over a period of a few hours (e.g., (Zimmerman & Kremer 1989)). The limited evidence available suggests that algal acclimation to growth temperature begins within several hours of being exposed to a change in temperature (Lynch & Thompson 1984; Mitchel *et al.* 1989). Therefore, algal flexibility in adaptation mechanisms as a response to temperature complicates the estimation of the optimal temperature range. This may explain why many investigators working with pure cultures have produced different temperature optima (Li 1980).

According to Kuebler *et al.* (1991) temperature has several effects on photosynthesis and the subsequent downstream biochemical reactions which result in algal cell growth. These can be divided into two categories: (1) short-term effects (less than one generation time; minutes to hours); and (2) long-term effects (more than one generation; more than one day). The response of photosynthesis to temperature is dependent upon the amount of light available, with the response at sub-saturating light levels being very different from that at saturating light levels (Geider 1987). The rate of photosynthesis will increase up to an optimum temperature, beyond which it declines rapidly. In some cases, the maximum photosynthetic rates occur over a range of several degrees rather than at a single fixed point of temperature (Oates & Murray 1983; Madsen & Maberly 1990). Verity (1981) showed that the rate of photosynthesis varied markedly with growth temperature under light limiting as well as under light-saturated conditions. An attempt to explain this was made by (Raven & Geider 1988) who concluded that the limiting processes in photosynthesis (and growth) at low photon flux densities are related to light absorption and the efficient transfer of the resulting excitation energy to operational reaction centres and downstream reactions.

Reasons for differences between the thermal relationships of growth and photosynthesis have been discussed by (Kuebler *et al.* 1991). Photosynthesis is not the sole factor regulating growth, and other aspects of metabolism such as the rate of dark respiration and leaking (efflux) of organic carbon will influence the amount of fixed carbon available for growth (Verity 1981). The reason for the discrepancy between

the effects of temperature on photosynthesis and on growth is the respective timescale of these processes.

2.4.7 Algal growth and temperature relationship

Algal species exhibit a positive relationship between temperature and metabolic activity by increasing growth rate with increasing temperatures up to an optimal temperature. Growth rates are highest at this temperature, and above it decline to zero, often rather rapidly. The minimum temperature (base temperature) is selected as the temperature at which photosynthesis approaches zero and is about 4°C.

The effect of temperature on algal growth is generally explained in terms of the temperature coefficients of reaction rates, which in turn depend on the activation energies of the reactions. The activation energy, A*, is the amount of heat energy, which has to be supplied to a reaction mixture in order to activate it.

Temperature characteristics for a particular organism remain constant only over a small temperature range (typically ± 10°C); the changes in activation energy indicate differences in rate-controlling reactions or in metabolic regulation (Nazaroff & Alvarez-Cohen 2001). The temperature dependence of a biological process is commonly characterised by the temperature quotient, Q_{10} by the following equation:

$$Q_{10} = \frac{k_2}{k_1} \tag{2.22}$$

where k_1 is the initial rate of reaction at T °C and k_2 is the rate of reaction at T+10°C.

The dependence of the reaction constant (k) upon temperature (T) is described by the Arrhenius equation (Sutcliffe 1977):

$$\ln k = \ln k_0 - \frac{A^*}{RT} \tag{2.23}$$

where constants k_0 the initial reaction constant and A^* the activation energy are determined empirically; R is the gas constant, and T is the absolute temperature (K).

Q_{10} relates to activation energy as follows:

$$\ln Q_{10} = \frac{A^*}{R} \times (\frac{1}{T} - \frac{1}{T+10}) \tag{2.24}$$

Eppley (1972) analysed the effect of temperature on algal growth in batch cultures and showed that maximum specific growth rate μ_{max} at their respective temperature optima had a Q_{10} (temperature coefficient of growth rate) of 1.88. Furthermore, the work outlined two common trends: Firstly, there is a gradual and exponential increase in μ with temperature up to 40 °C; and secondly, at values of specific growth rate μ below 40 °C values appeared to fall within an envelope that could be described by an empirical equation:

$$\mu = 0.851(1.066)^T \tag{2.25}$$

where μ is the algal specific growth rate (h^{-1}) and T is temperature (°C).

According to Eppley (1972), freshwater and marine algal species grown as laboratory pure cultures show very close values of growth rate μ with respect to variations in temperature, despite big differences in their environment. Goldman and Carpenter (1974) undertook a similar analysis for microalgae growing in chemostat cultures, and deduced Q_{10} values of 2.08-2.19, in good agreement with values of Q_{10} of approximately 2.0 from (Kremer 1981) and (Sukenik *et al.* 1987).

An Arrhenius-type equation to describe growth rates was also adopted by Goldman (1977; Goldman 1979), who used combined data on growth rates of freshwater and marine algal species to derive the following equation:

$$\mu = 0.59(1.066)^{2T} \tag{2.26}$$

where the specific growth rate, μ, is in units of day^{-1}, and T is the temperature in °C.

In wastewater practice the influence of temperature on biological reactions is often expressed as follows (Tchobanoglous & Burton 1991):

$$r_T = r_{20}\theta^{(T-20)} \tag{2.27}$$

where r_T is the reaction rate at T °C, r_{20} is the reaction rate at 20 °C, θ is temperature-activity coefficient, and T is the temperature in °C; thus the temperature effect is expressed in comparison with growth at 20 °C.

In their natural environment, algae are often under limitation from light intensity or nutrient deficiency in addition to suboptimal temperatures. Thus, temperature is seldom the sole reason for limited growth. Figure 2.6 shows the general response of algal growth rate to light intensity at various temperatures. As growth temperature falls, the light saturation of growth occurs at lower light intensities, which is due rather to a decrease in the temperature-dependent μ_{max} than to a change in the intrinsic properties of photochemical reactions. There is a noticeable similarity between the temperature-dependent growth curve and the *PI* curve.

Therefore, the interaction between temperature and light on algal growth rate shows that with decreasing temperatures light saturation occurs at lower light intensities.

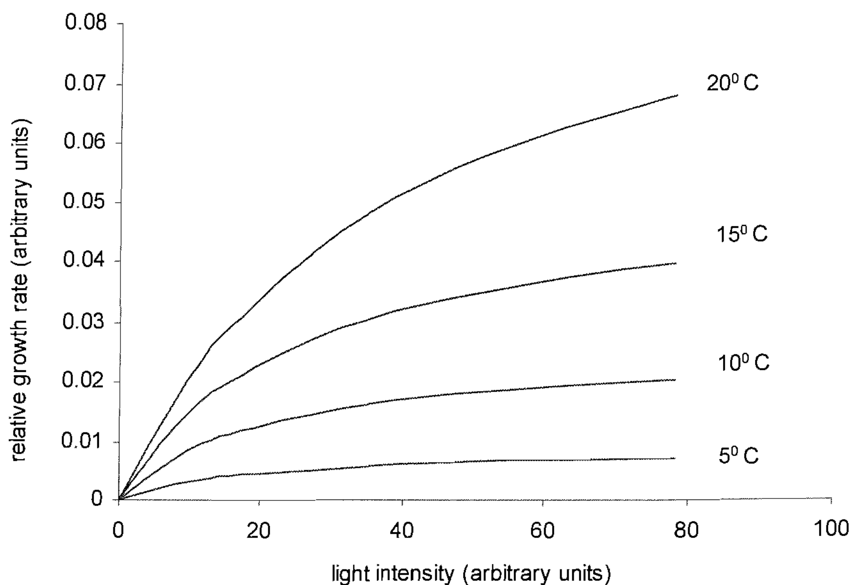


Figure 2.6 Effect of temperature and light intensity on relative growth rate of an algal species.

2.4.7.1 Low temperatures and algae

Temperature extremes impose stresses of variable severity that depend on the rate of temperature change, its intensity and duration. Temperature optima for growth of many plants depend directly on the temperature range of their natural environment.

For mesophilic plants the temperature optima lie in the range 18-25 °C. In terms of growth, however, the algae are able to adapt to a range of suboptimal temperatures within particular limits, which are thought to be determined by a species-specific genotypic allowance that is yet not well understood (Raven 1988).

Among mesophilic plants two kinds of low temperature stress are common: chilling at temperatures above 0 °C and freezing at temperatures below zero. Additionally most plant species, including those from temperate climates, are generally frost-sensitive or develop frost tolerance only upon hardening.

It has frequently been suggested that the primary site of freezing injury in plant cells is the cellular membrane (Heber 1968; Mazur 1969; Yoshida & Sakai 1974). An increase in phospholipids, which are known to be essential components of cellular membranes, has been observed during hardening of plants (Yoshida & Sakai 1973; Siminovitch *et al.* 1975; Willemot 1975). Furthermore, development of frost hardiness in plants is intrinsically associated with alterations of metabolic patterns such as augmentation of protein synthesis, increase in concentration of carbohydrates, lipids etc (Siminovitch *et al.* 1968; Mazur 1969; Hatano *et al.* 1976).

Plant sensitivity towards low temperature, and development of chilling and frost hardiness involve three vacuolar events related to V-ATPase activity (V-ATPase is the dominate H^+-pump at endomembranes of most plant cells) (Yoshida *et al.* 1999). One of the primary events of chilling injury appears to be an inhibition of V-ATPase activity; as a consequence, the formation of pH gradients is inhibited and probably compartmentation of solutes is disturbed. Finally, the fluidity of membranes has to be adjusted to low temperatures by an increase of the membrane content of unsaturated fatty acids.

The low temperature limitation of electron transport or carbon fixation reduces the ability of the plant to use light, and the resulting excess light energy may cause photoinhibition (Moll & Steinback 1986). This has been studied extensively in chilling sensitive higher plants and has been documented in unicellular algae (Falk *et al.* 1990).

Despite the damage due to the effect of low temperatures some algal cells can survive severe conditions and flourish after. For marine phytoplankton several possible

mechanisms have been proposed to explain survival under adverse conditions of temperature and light, including the formation of resting stages or spores (Anderson 1976), the use of heterotrophic modes of nutrition (Harris 1978; Deventer & Heckman 1996), and decreased cellular metabolism (Antia & Landymore 1974; Smayda & Mitchell-Innes 1974; Dehning & Tizler 1989). Some species of diatom are known to form cyst stages that survive for long periods of darkness and low temperature. Resting stages may not be distinguishable morphologically but instead may undergo biochemical and/or physiological changes (Hargraves & French 1975; Antia 1976). No evidence was found to indicate that *Chlorella vulgaris* or *Scenedesmus subspicatus* could produce resting cysts; however, a physiological resting stage may be a possibility (Hatano *et al.* 1976).

2.5 Algal growth in batch cultures

Surprisingly little research has been directed towards the participation of green algae in the spring bloom event. Studies with other groups of algae indicate that that the bloom is developed from a small number of cells that survive the harsh winter conditions. The spring algal boom usually has one predominant species and its growth resembles growth in a culture of limited volume (Fogg 1975; Abeliovich 1986; Richmond 1986), i.e. batch culture in the laboratory.

2.5.1 Algal growth in limited volume culture

Laboratory culture in limited volume comprises a limited volume of medium containing the saturation concentrations of necessary nutrients, inoculated with a number of cells and exposed to suitable conditions of light and temperature (Fogg 1975). The batch growth cycle can be divided into phases depending on whether the algal biomass, expressed as weight or cell numbers, is increasing, constant or falling (Figure 2.7).

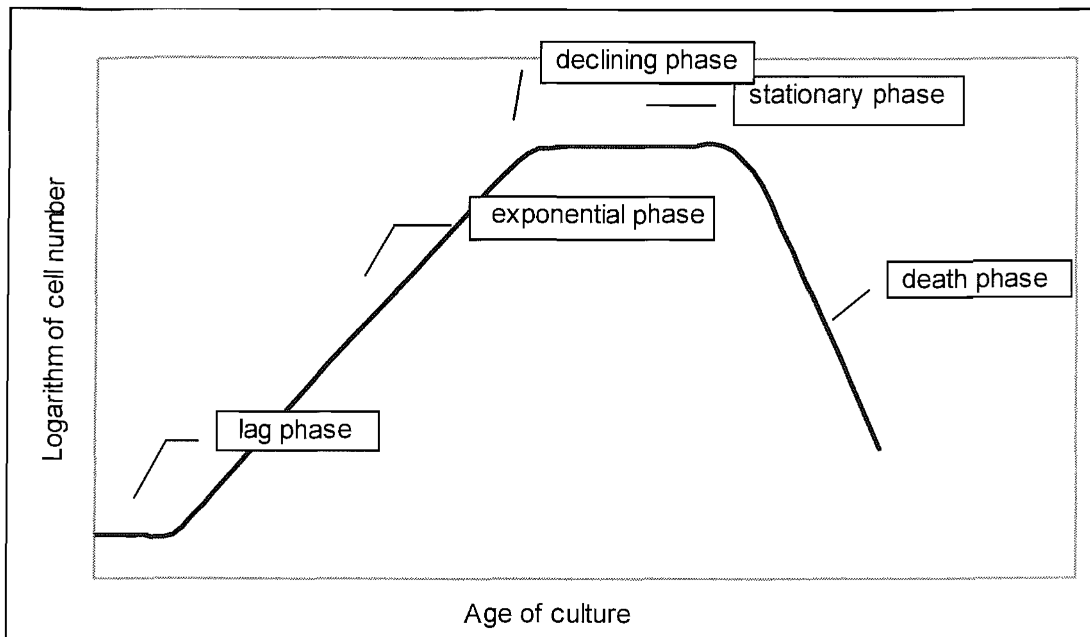


Figure 2.7 Schematic representation of algal growth in culture of limited volume (i.e., batch culture).

Thus, the following phases can be recognised:

1) lag phase;

2) exponential phase;

3) declining phase;

4) stationary phase; and

5) death phase.

The lag phase of growth is generally recognised as the phase during which the inoculum cells adapt to the medium and conditions and the culture shows no increase in growth.

If growth of an algal cell is not limited by external factors or if these external factors can be kept constant during growth, an exponential growth of the population will occur. This arises from a more general description of the growth of the population: the rate of growth is proportional to the number of cells at any particular time. During the

exponential (logarithmic) growth phase the biomass of population doubles over equal time intervals. The growth rate for the entire exponential phase is therefore constant.

There is ample evidence that the shape of the growth curve for a population in a batch culture is fixed, i.e. is predetermined by the biological nature of the system, but the parameters of the curve are a function of light intensity and other factors (Myers 1962; Droop 1969; Stein 1973).

From the nature of exponential growth, it follows that the absolute amount of growth in any mean generation time is equal to the total in the period, however long, that has gone before (Fogg 1975). Hence, the reduction of the concentration of a nutrient from a level saturating for growth to zero is abrupt. Eventually in a culture of limited volume, exponential growth must cease.

Examples of factors involved in the transition to the declining phase are given by different researchers: exhaustion of nutrients such as nitrate, iron (Fogg 1975); rate of supply of carbon dioxide (Azov *et al.* 1982); alteration of pH of the medium as a result of preferential absorption of particular constituents from the medium (Becker 1994); reduction of the light intensity by self-shading (Richmond 1986); and autoinhibition by excreted metabolites (Fogg 1953). The duration of the period of declining of relative growth depends on the nature of the limiting factor. Nutrient exhaustion or autoinhibition usually results in an abrupt transition from the exponential to the stationary phase (Fogg 1975), but if light is limiting, a prolonged phase of linear growth may intervene (Richmond 1986).

During the stationary phase, a steady concentration of cell mass per unit volume of cell suspension is maintained as losses due to catabolism are balanced by anabolic processes (Sorokin 1973).

2.5.2 Relationship between algal growth and growth limiting factor

Among the limitations on growth in culture are the culture temperature, the nutrient supply, and the supply of photons for photosynthesis. For a given species, the relative growth rate is a function of temperature, light intensity, and other environmental factors.

In a limited volume of culture there is a steady decline in the nutrient supply per algal biomass unit. The growth rate of an algal culture can be considered as a function of the limiting concentration of a nutrient, S, and is given by the Monod equation (Monod 1949):

$$\mu = \mu_{\max} \frac{S}{(K_S + S)} \tag{2.28}$$

where μ is the specific growth rate of a given algal species; S is the concentration of limiting nutrient; μ_{max} is the maximum growth rate of the species when growth conditions are ideal and a higher growth rate cannot be induced, and K_s is the constant numerically equal to the concentration of the nutrient giving half of the maximum growth rate. At low values of S, the Monod equation approximates a first-order equation in which the specific growth rate is linearly related to the concentration of the limiting nutrient:

$$\mu = \mu_{\max} \frac{S}{K_S} \tag{2.29}$$

When $S >> K_S$, an approximately zero order relationship is observed:

$$\mu = \mu_{\max} \tag{2.30}$$

and the specific growth rate is at maximum and no longer depends on the concentration of the varied nutrient, but rather on the fixed environmental conditions such as light and temperature (Figure 2.8).

A nutrient is limiting only when S is not large compared with K_S. It follows from this relationship that as long as cell numbers are sufficiently low so as not to alter appreciably the concentration of the nutrient in the batch culture, μ will remain constant during batch growth even though the concentration may be limiting. For example, K_s for nitrate uptake was found by (Eppley *et al.* 1969) to be positively correlated with cell size, with generation time, and with K_s for ammonium uptake.

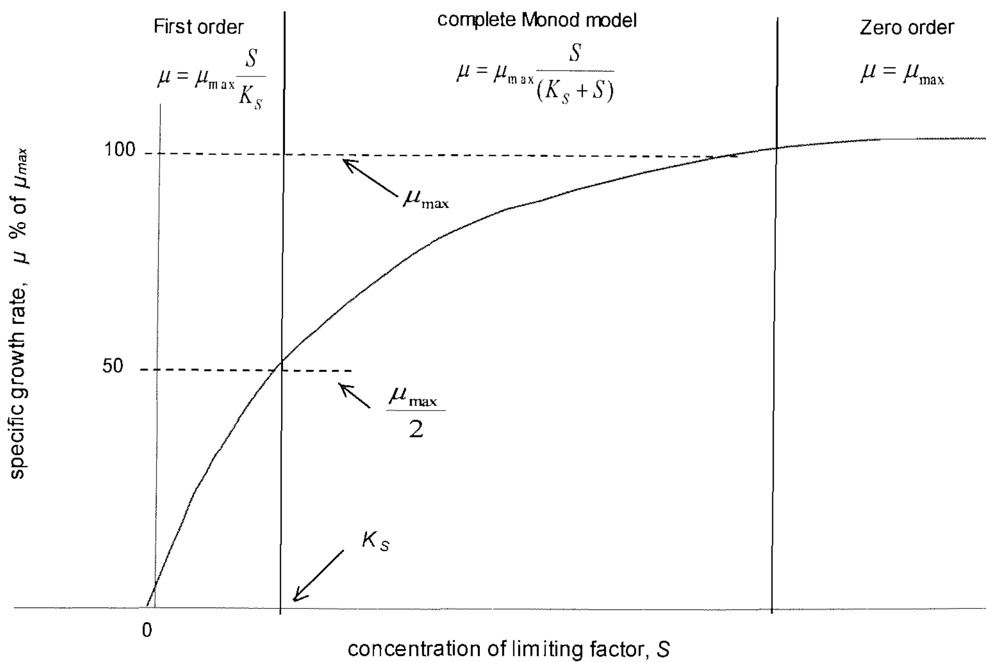


Figure 2.8 Relationship between growth limiting factor and specific growth rate for the Monod model (after (Goldman *et al.* 1974).

The relationship of relative growth rate to nutrient concentration is, however, more complicated than the above simple hyperbolic expression suggests. Under conditions where adequate light and nitrogen are present, the growth rate of algae will be limited by the amount of inorganic carbon available. There is uncertainty, however, as to which species of inorganic carbon should be considered as substrate in the Monod equation. As discussed earlier, some investigators suggest that total inorganic carbon should be used (Goldman & Carpenter 1974), whereas others argue that dissolved carbon dioxide is more appropriate (King 1970; King & Novak 1974). The latter approach has the advantage that it will predict the decrease in algal growth rate that is observed at high pH, as a result of the shift in the carbonic acid equilibrium towards more bicarbonate and carbonate, and less CO_2.

Phosphate and other nutrients may be accumulated in excess (Fogg 1953), and thus the relative growth rate of an alga may not respond at once to a change in the external concentration of these nutrients. The response to environmental factors such as light and temperature is immediate, however (Geider 1987). Light could be also considered as a growth limiting factor and the limitation being described by the Monod equation.

Much of the available data shows that unicellular algae growth rates are similar for marine and freshwater species, as is their variation in specific growth rate with temperature. Hence both data pools can be used for algal growth estimation.

Chapter 3 MATERIALS AND METHODS

3.1 Experimental cultures and media

Cultures of *Scenedesmus subspicatus* CCAP 276/20 (Figure 3.1) and *Chlorella vulgaris fo. viridis* CCAP 276/20 (Figure 3.2) were obtained from the Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, Ambleside, UK.

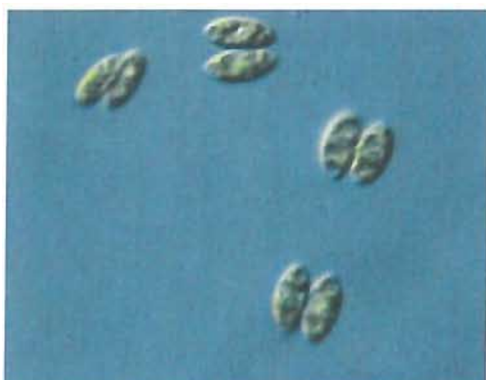


Figure 3.1 Morphological appearance of *S.subspicatus* (scale: 10μm ≅ 5cm)

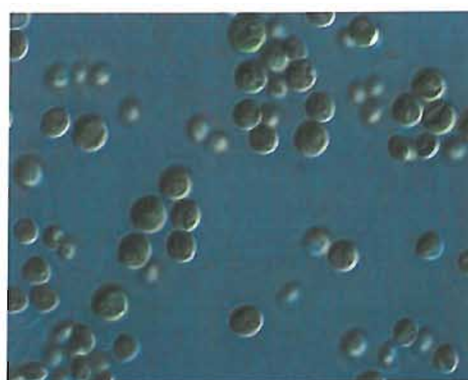


Figure 3.2 Morphological appearance of *C.vulgaris* (scale: 10μm ≅ 7cm)

Mineral Jaworski's Medium (JM) was used as a basic growth medium for experiments and algal stock maintenance. The recipe of original MJ is given in Table 3.1. The molar C:N ratio in the JM was calculated to be 1:6 indicating that the majority of carbon sustaining algal growth is derived from atmospheric CO_2.

Table 3.1 Original chemical composition of Jaworski's Mineral Medium.

Chemical component*	Concentration (mg l^{-1})
1. $Ca(NO_3)_2 \cdot 4H_2O$	20.0
2. KH2PO4	12.4
3. $MgSO_4 \cdot 7H_2O$	50.0
4. $NaHCO_3$	15.9
5. EDTAFeNa	2.25
$EDTANa_2 \cdot 2H_2O$	2.25
6. H_3BO_3	2.48
$MnCl_2 \cdot 4H_2O$	1.39
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.0
7. Cyanocobalamin	0.04
Thiamine HCl	0.04
Biotin	0.04
8. $NaNO_3$	80.0
9. $Na_2HPO_4 \cdot 12H_2O$	36.0

*9 groups of chemicals were prepared, sterilised by filtration (Whatman, GF/C filter), and stored (+4°C) separately as stock solutions (in concentration of $>10^3$ times).
For solid JM 15g l^{-1} of bacteriological agar (Oxoid L11) was added.

3.1.1 Modifications of the growth medium for nutrient uptake experiments

Prior to experiments looking at the influence of physical environmental factors such as light intensity and temperature on algal cells, the medium was modified in series of three experiments to give growth-saturated concentrations of primary nutrients (C, N, P) as follows:

Experiment 1: Carbon enrichment experiment

Sodium bicarbonate ($NaHCO_3$) concentrations were increased to give a range of inorganic carbon concentrations from 0.92 to 18.49 mmol l^{-1}. The chelating

agents EDTAFeNa and $EDTANa_2$ also contain traces of organic carbon, but these were considered to be non-bio degradable by algae.

Due to the high bicarbonate concentrations used the pH values of the medium increased (pH = 7.2 at 0.92 mmol C l^{-1} and pH=7.9 at 18.49 mmol C l^{-1}), but to reduce the risk of CO_2 escaping they were not corrected.

To check that the rise in pH did not cause precipitation of anions (HPO_4^{2-}), the pH value of pure modified culture medium was sequentially raised using NaOH 1M. After each increase in pH a sample of the well-mixed medium was centrifuged (at 0.7 g), stored and analysed for anions. No precipitation was found over the range of values encountered in the experiment. The modified medium was sterilised by filtration (Whatman, GF/C filter).

Experiment 2: Nitrogen uptake experiment

The medium was modified to give a range of inorganic nitrogen concentrations from 0 to 4.56 mmol l^{-1}. This was achieved by altering the original nitrogen source as follows:

1) To retain a constant concentration of calcium the calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot \times 4H_2O$ was replaced by calcium chloride ($CaCl_2$).

2) The concentration of $NaNO_3$ was recalculated to give the desired concentration of NO_3^- ions and used as the sole source of nitrogen in the medium.

3) The NH_4^+ present in the medium as $(NH_4)_6Mo_7O_{24} \cdot \times \cdot 4H_2O$ (0.005mmol) was not considered to contribute greatly to the nitrogen pool and was treated as negligible.

Experiment 3: Phosphorus uptake experiment

The medium was modified to give a range of inorganic phosphorus concentrations from 0 to 0.58 mmol l^{-1}. The composition was changed as follows: di-sodium hydrogen orthophosphate 12-hydrate $Na_2HPO_4 \cdot 12H_2O$

was omitted, and the concentration of potassium phosphate anhydrous KH_2PO_4 was recalculated to give the required concentration of P-source.

3.1.1.1 Radioactive tracer experiments

Cultures (100 ml) used in radioactive tracer experiments were inoculated with $H^{14}CO_3^-$ (6.21µCi) using pipette (Finnpipette, USA) in designated area (fume cupboard), carefully labelled and placed in orbital incubator with preset experimental conditions. The working area was thoroughly checked on radioactive losses or spills using (Geiger counter). Records of isotope in stock and quantities disposed were recorded on a provided clipboard. At selected time intervals, samples (5 ml) of the ^{14}C labelled algal suspensions were filtered onto a glass fibre filter (Whatman, GF/C). The collected algal biomass was rinsed with 20 ml of HCl 0.01M solution, suspended into 15 ml of scintillation cocktail (Optyphase, Wallac), left for around 24 hours to react with the scintillation cocktail, and assayed for the amount of radioactive carbon. All manipulations with radioactively labelled algal cultures and subsequent storage of the samples and wastes were carried out in designated and clearly labelled areas (fume cupboard, working bench area, glassware, equipment).

3.1.2 Preparation of algal cultures for experiments

Algal cultures were maintained on semi-solid agar - JM medium for long-term storage. For sort-term algal cells were inoculated into test tubes with sterile liquid JM and allowed to grow for 1-2 weeks. The cultures were then resuspended in 500 ml of original or modified JM in (1-litre Erlenmeyer flasks), placed into an orbital incubator (SANYO, Japan) at a constant illumination of 78.3 µmol $m^{-2}s^{-1}$, 20 °C and 100 rpm to allow algae reach the stationary phase of growth. This ensured that the cultures were in good physiological condition at the start of each experiment. Microscopic inspection also confirmed that the cultures had a normal morphology.

Algal cells were harvested by centrifugation for 15 minutes in Nalgene centrifuge bottles (150 ml) at approximately 1750 g (WIFUG 4000E). The old medium was discarded and the algal biomass was resuspended in freshly prepared medium modified as appropriate for the experimental conditions. Homogeneity of the algal

suspension with the medium was obtained, and initial OD_{678} readings were taken before each experiment. Initial values of pH were also determined.

3.2 Sample preparation and storage

Samples for OD_{678} readings were collected at selected time intervals and were read immediately. To prepare samples for anion inorganic carbon analysis, they were and centrifuged at 14000 rpm for 20 minutes (Eppendorf) and then stored in a freezer at –20 °C for up to 3 weeks until analysis was carried out.

3.3 Measurements

Optical density of algal cultures was measured in micro-cuvettes (Kartel) at $\lambda = 678$ nm using a Cecil (3000 series) Scanning Spectrophotometer.

Measurements of pH were made using a JENWAY 3010 pH meter standardised with buffers at pH 7 and 9 (Russell).

Oxygen concentrations in the flask headspace were measured by gas chromatography (GC) (Varian, CP-3800) using a chrompack capillary column (Varian) at 50 °C, with peak detection by Thermal Conductivity Detector. Argon was used as the carrier gas at a flow rate of 6 ml min^{-1}. Samples of 25ml each were manually injected through a gas injection loop. Standardisation was by a commercial gas mixture SCOTTY II (mix 218) containing CO_2, CO, H_2, CH_4, O_2, and N_2 (Supelco, UK Ltd).

Initial oxygen concentrations in the flask's liquid phase were measured by YSI 550A Dissolved Oxygen Meter.

Anions were measured by ion chromatography using a DIONEX DX-500 with electrochemical detector ED40 operating in conductivity mode. An AS9-SC anion column was used in conjunction with an anion self-regenerating suppressor ASRS-1. Carbonate buffer (1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ solution) was used as an elluent and the flow rate was adjusted to 2 ml min^{-1}.

Concentrations of Dissolved Inorganic Carbon (DIC) were measured using a carbon analyser (Dorhmann DC-190, Rosemount Analytical). Standardisation was performed with bicarbonate ($NaHCO_3$) solutions.

Algal respiration was measured using a Rank Respirometer (Rank Brothers Ltd.) with a 5 ml respirometric chamber. Standardisation was performed using oxygen depleted (sodium bisulphite, N_2(gas)) and oxygen saturated distilled water.

Where ^{14}C was used in experiments, the concentration incorporated into algal biomass was measured by WALLAC scintillation counter (1414 WinSpectral™) with internal reference library.

Irradiance in the orbital incubator used in growth experiments was standardised using a LI-210SA Photometric sensor (LI-COR, USA) in conjunction with a data acquisition system (KEITHLEY 2700 Multimeter).

Dry weight (at 103 °C) and chlorophyll **a** (acetone extraction) tests were performed according to Standard Methods (APHA 1995).

Microscopic examination of algal cells was performed with Diaplan (Leitz) microscope at × 40 magnification (Lenz, Wetzlar).

Algal cells were counted in improved 'Neubauer' haemacytometer (depth: 0.02 millimetres). The number of algal cells was calculated in 80 squares (where each square has area of $0.0025 mm^2$) using Williams and Shaw (1976). The volume of 1 square is:

$0.0025 mm^2 \times 0.002 mm = 0.00005 mm^3$;

The volume of 80 squares is 0.004 (mm^3).

Thus, the amount of cells in

$$1 \text{ mm}^3 = \frac{n}{0.004} \tag{3.1}$$

where n is an amount of cells counted on 80 squares.

Plate count (surface spreading technique) was used to assess a viability of algal cells. Algal suspensions underwent a series of dilution to reach 1×10^{-4} of original cell density. 0.1 ml of the diluted suspension was inoculated onto JM agar plates. The plates were incubated at room temperature for 7 days under continuous illumination (approximately 80 μmolm^{-2}s^{-1}). The number of colonies developing was counted and each was assumed to represent a single viable cell.

3.4 Experimental design

3.4.1 Calibration between growth parameters

Experiments were carried out to determine factors affecting the relationships between dry weight, chl **a** concentration, cell numbers and OD_{678}. Cultures of *C. vulgaris* and *S. subspicatus* were grown in batch cultures at six different light intensities. To obtain initially dense algal suspension the cell biomass from exponentially growing cultures was concentrated by gentle centrifugation (250 rpm for 7 min). Dilutions of algal suspensions were prepared at the following concentrations: 1, 1/2, 1/4, 1/8, 1/16, and 1/32. To eliminate possible dilution errors, the concentrations of algal biomass were kept constant by increasing the volume of processed samples (e.g. the volume of sample processed for the initial dilution was 5 ml, for the 1/2 dilution the volume of processed sample was 10 ml, for 1/4 dilution 20 ml etc.). Measurements of OD_{678}, dry weight, chl **a** and cell counts were taken at each dilution. Dry weight, chl **a** content and cell counts were plotted against OD_{678} and the calibration coefficient was calculated from the slope of a line of best fit.

3.4.2 Experiments on algal nutrient requirements

A number of experiments were conducted to look at the effects of nutrient concentration on algal growth. All experiments were carried out with batch cultures of *S. subspicatus* and *C. vulgaris* at constant light intensity (78 μmol m^{-2}s^{-1}), temperature (20 ^{o}C) and agitation.

3.4.2.1 Carbon

Flasks containing an initial volume of 100 ml of modified culture medium were set up in triplicate. Control incubations in modified growth medium without algae were carried out simultaneously for each set of experiments. The sampling time intervals and the duration of the experiments were dependent on the onset and the duration of the exponential phase. A gas sample of 25 ml was taken from the headspace of each flask before it was opened to collect the liquid samples ("Effects of carbon enrichment on algal growth and activity" experiment only). Algal growth was followed by measuring OD_{678} of algal suspensions; nutrient depletion by ion chromatography and dissolved inorganic carbon measurement. Figure 3.3 shows the experimental apparatus used for the experiments, consisting of an Erlenmeyer flask (250 ml) sealed with a modified silicon plug for gas accumulation and sampling in the flask headspace.

Figure 3.3 Experimental apparatus for carbon enrichment experiment.

3.4.2.2 Experiments to investigate the effects of pH on growth rates

An experiment was carried out to assess the effect of air CO_2 presence on air/water surface of experimental apparatus on durations of exponential phase of growth and rates of growth of *S. subspicatus* and *C. vulgaris* cultures. Algal cultures were grown in modified JM medium with 0.18, 1.85, 9.25 and 18.49 mmol C l^{-1}. One set of flasks was left open to air contact (variant 1) while another set was sealed by rubber stoppers (variant 2) during entire experiment (with exclusions on short periods of sampling). Algal cultures preparation were made as described in section 3.1.2. Measurements of OD_{678} and pH changes were recorded every 8 hours during the experiment. Experimental variants were performed in triplicates.

3.4.2.3 Nitrogen and Phosphorus

Sampling was carried out at eight-hour intervals over a period of 72 hours. Algal growth was followed by measuring OD_{678} and nutrient depletion by ion chromatography and dissolved inorganic carbon (DIC) measurement. Flasks containing an initial volume of 100 ml of modified culture medium were set up in triplicate.

3.4.3 Light - temperature interaction experiment

Algal growth, rates of nutrients uptake and photosynthetic oxygen production were tested in batch cultures at the following combinations of irradiance and temperature: 78.3, 62.7, 47.0, 31.3, 15.7 and 7.8 μmol $m^{-2}s^{-1}$, and 5, 10, 15, and 20 °C, respectively. Hence, for each species a set of 24 experiments was performed.

Algal stock cultures were pre-incubated at the experimental light intensity and temperature for 48 hours prior to the experiment and prepared for the experimentation following the procedure described in section 3.1.2.

Flasks containing an initial volume of 100 ml of algal biomass suspended in the modified culture medium were set up in four replicates. Algal growth was followed

by measuring OD_{678} and nutrient depletion by ion chromatography. Sampling was carried out at eight-hour intervals over a period of 80-104 hours.

To assess photosynthetic carbon uptake each combination of light/temperature/ algal species was subdivided into 'cold' (without addition of ^{14}C) and 'hot' flasks (with addition of ^{14}C), with a further subdivision of both types into 'light' (algal cultures exposed to light) and 'dark' variants (algal suspensions kept in complete dark for the whole duration of the experiment). 'Control' variant was added to ‘hot’ ‘light’ variants (algal suspension inhibited by addition of chloroform (99%) and exposed to light) to detect any side carbon uptake activity.

At incubation temperatures 15 and 20 °C the variants were sampled every hour, but at incubation temperatures 5 and 10 °C every 2 hours. All samples (5 ml) were analysed for ^{14}C uptake. 'Cold' variants were sampled immediately after ‘hot’ and OD_{678} readings were taken to provide with a record on a progress of algal growth. Total experimental time was 8 hours.

3.4.4 Respiration experiment

In the first experiment cultures of *S. subspicatus* and *C. vulgaris* were grown at temperatures of 5, 10, 15, 20 °C at 78.32 $\mu mol\ m^{-2}s^{-1}$ of irradiance for 48 hours. In the second experiment 6 different light intensities were used, of 78.32, 62.7, 47.0, 31.3, 15.7, 7.8 $\mu mol\ m^{-2}\ s^{-1}$ at 20 °C for 48 hours. Measurements of algal dark respirations were taken by recording oxygen depletion from dense algal suspension (5 ml).

3.4.5 Algal survival at low temperatures

Cultures were prepared for low temperatures by the hardening process proposed by Hatano (1976). *S. subspicatus* and *C. vulgaris* cultures were grown at room temperature (20 ±2 °C) and ambient light intensity until they reached a stationary phase of growth. The hardening was performed in the orbital incubator at +8 °C and constant illumination of 78.3 $\mu mol\ m^{-2}s^{-1}$ for 48 hours. Algal biomass was concentrated using a combination of sedimentation and low-speed centrifugation, then

resuspended in freshly prepared medium to the biomass concentration of 231.4 and 302.1 mgl^{-1} DW for *C. vulgaris* and *S. subspicatus,* respectively. Algal suspension was then distributed into 150 ml polystyrene containers (total: 100 containers). The cultures were kept stationary in complete darkness at +4 °C (group 1) and -20 °C (group 2).

At 7-day intervals a single container of each species from both groups was retrieved from the storage conditions. The cultures were warmed up by following methods: leaving the cultures outside the refrigerator till it reached a room temperature (group 1), and by thawing in a water-bath at +30-35 °C for group 2. 1ml from each variant was taken to prepare a serious of dilutions for algal plating. Cultures were centrifuged at 1750 g, resuspended in 2 litres of freshly prepared modified JM. The algal suspensions (100ml) were distributed into Erlenmeyer flasks and incubated at 25 °C and 62.7 $\mu mol\ m^{-2}s^{-1}$ of constant illumination for 24 hours to assess growth response and photosynthetic ^{14}C uptake (the growth conditions were chosen from the set of preliminary experiments) Thus, the viability of each culture in both groups was determined by plate counts, growth response (OD_{678}), ^{14}C uptake, increase in dry weight, and chl **a** content.

3.4.6 Statistical considerations for experimental design

For batch cultures replicates were analysed where each Erlenmeyer flask served as one replicate. Blanks were prepared similarly but without algal cells in the flasks. Experimental results of each single experiment are reported as an average ± standard deviation (Appendix C). Graphs are plotted using an average values calculated from the replicates with error bars indicating a scattering in values among replicates. Results from several experiments are reported as an average ± standard error (SE).

Results were combined and analysed together by one-way analysis of variance (ANOVA) at $P \leq 0.05$, by Student's t-test at $P \leq 0.05$, and correlation coefficient test using Excel 2000. Linear regression analysis between the observed values was used to verify the fitting quality according to Laws and Archie (1981).

3.5 Data calculations

The following equations and definitions were used in interpretation of results.

3.5.1 Algal growth rate

Exponential growth rate of algal culture can be presented as the number of doublings of cell material over a specified time interval. The principal expression for exponential growth rate (μ) then as follows:

$$\mu = \frac{\log_2 X_1 - \log_2 X_0}{t_1 - t_0} \tag{3.2}$$

where X_0 and X_1 are the numerical values of two subsequent optical density at λ= 678nm (OD_{678}) readings, and t_0 and t_1 are the corresponding times (h). Determinations of exponential growth rate values of algal cultures placed under experimental conditions were made on the basis of calculations for optical density proposed by Sorokin (1973). The detailed example of calculations is given in Appendix B (section B.1).

To obtain the maximum growth rate (μ_{max}) and half-saturation constants (K_S) of species in relation to growth limiting factors such as inorganic carbon, nitrogen phosphorus and light. The experimental data for specific growth rates were fitted into the Monod equation (2.28) by running through 'solver' option of Microsoft Excel (Appendix B, section B.1).

Temperature influence on algal growth was estimated by employing the equation (2.27) from (Tchobanoglous & Burton 1991).

3.5.2 Nutrient uptake calculations

The uptake rate (V) was calculated from the change in nutrient concentration (bicarbonate, nitrate, or phosphate) in the medium during exponential growth, using the equation from Pedersen (1994):

$$V = \frac{[(S_0 \times vol_0) - (S_t \times vol_t)]}{(t \times B)} \quad (3.3)$$

where S_0 is a nutrient concentration, and vol_0 is the medium volume at the beginning of the exponential phase, S_t is a nutrient concentration and vol_t is the medium volume at the end of the exponential phase, t is the time elapsed between the beginning and the end of the phase, and B is the biomass dry weight (DW). Results of the calculations were expressed as μmol mg^{-1} h^{-1} DW after accounting for the uptake rates of the controls.

The experimental data for each nutrient uptake were fitted into the Michaelis-Menten equation (2.6) by running through 'solver' option of Microsoft Excel (the detailed example of calculations is given in Appendix B).

3.5.3 Photosynthesis rate

3.5.3.1 Gas chromatography

Oxygen accumulation in a flask's headspace was calculated by employing Henry's law which states that the concentration of gaseous species dissolved in liquid phase is proportional to the species concentration in the gas phase. The stated relationship can be expressed in following form:

$$C_w = K_{H,g} P_g \quad (3.4)$$

or

$$P_g = H_g C_w \quad (3.5)$$

where C_w is the equilibrium species concentration in the aqueous phase (M); P_g is the equilibrium partial pressure of the species in the gas phase (kPa); $K_{H,g}$ is the form of Henry's law constant (M kPa^{-1}); and H_g is another form of Henry's law constant, with units of kPa M^{-1} (the detailed example of calculations is given in Appendix B).

3.5.3.2 Radioactivity

Inorganic carbon uptake was calculated from ^{14}C uptake on the basis of an equation:

$$\frac{^{14}C_{fixed}(a)}{^{14}C_{available}(b)} = \frac{^{12}C_{fixed}(c)}{^{12}C_{available}(d)} \tag{3.6}$$

where (a) is measured from radioactivity collected on filters, (b) is the amount of ^{14}C added to each bottle, (c) is the primary production, and (d) is the unlabelled metabolically available carbon.

The rate of photosynthesis (P) was calculated based on ^{14}C uptake using the equation from Parsons *et al.*(1984):

$$P = \frac{(R_S - R_B) \times W}{R \times N} \tag{3.7}$$

where R is the total activity (dpm) of labelled bicarbonate added, N is the number of hours of incubation, R_S is the sample activity (dpm), R_B is the dark bottle activity (dpm), and W is the total weight of carbon present (mg l^{-1}).

Photosynthesis versus light intensity parameters (*PI* curves) were analysed using equation (2.8) from Jassby and Platt (1976).

Chapter 4 EXPERIMENTAL RESULTS

4.1 Calibration experiments

Cultures of *C. vulgaris* and *S. subspicatus* were grown at six different light intensities. Measurements of OD_{678}, dry weight, chl **a** and cell counts were taken at each dilution and conversion coefficients calculated for OD_{678}.

4.1.1 Dry weight

4.1.1.1 C. vulgaris

Conversion coefficients from two independent experiments are given in Table 4.1. The value of 269.0 ± 6.1 (±SE) derived as an average for both tests was used as the conversion coefficient for further calculations. The average value for the group "calibration 1" was 261.5 ± 7.7 (±SE) and for the group "calibration 2" was 276.5 ± 9.2 ((±SE). Comparison of the two groups by ANOVA gave a P-value of 0.203, which suggests that they are not significantly different.

4.1.1.2 S. subspicatus

Conversion coefficients from two independent experiments are given in Table 4.2. The value of 350.1 ± 9.6 (±SE) derived as an average for both tests was used as the conversion coefficient for further calculations. The average value for the group "calibration 1" was 325.7 ± 11.3 (±SE) and for the "calibration 2" was 374.4 ± 6.6 (±SE). Comparison of the two groups by ANOVA gave a P-value of 0.160, which suggests that they are not significantly different.

Table 4.1 Conversion coefficients for dry weight (mg l^{-1}) and OD_{678} of *C. vulgaris* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	262.9	0.996	275.6	0.995
15.7	262.8	0.997	277.7	0.995
31.3	268.6	0.995	294.3	0.998
47.0	255.9	0.999	296.8	0.996
62.7	288.5	0.998	234.2	0.998
78.3	230.6	0.998	280.6	0.999
Average	261.6		276.5	
± SE	7.7		9.2	

Table 4.2Conversion coefficients for dry weight (mg l^{-1}) and OD_{678} of *S. subspicatus* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	307.4	0.999	347.7	0.998
15.7	302.5	0.997	383.6	0.999
31.3	327.5	0.998	389.4	0.997
47.0	326.1	0.998	370.3	0.999
62.7	378.5	0.995	366.4	0.998
78.3	312.3	0.997	389.0	0.999
Average	325.7		374.4	
± SE	11.3		6.6	

4.1.2 Concentration of chlorophyll a

4.1.2.1 C. vulgaris

Conversion coefficients from two independent experiments are given in Table 4.3. The value of 2.25 ± 0.05 (±SE) derived as an average for both tests was used as the conversion coefficient for further calculations. The average value for the "calibration 1" was 2.17 ± 0.06 (±SE) and for the "calibration 2" was 2.32 ± 0.07 (±SE). Comparison of the two groups by ANOVA gave a P-value of 0.137, which suggests that they are not significantly different.

Table 4.3 Conversion coefficients for chl **a** content (mg l^{-1}) and OD_{678} of *C. vulgaris* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	1.94	0.999	2.33	0.993
15.7	2.23	0.998	2.12	0.999
31.3	2.03	0.996	2.21	0.997
47.0	2.22	0.998	2.56	0.987
62.7	2.28	0.987	2.24	0.990
78.3	2.34	0.998	2.47	0.992
Average	2.17		2.32	
± SE	0.06		0.07	

4.1.2.2 S. subspicatus

Conversion coefficients from two independent experiments are given in Table 4.4. The average value for the "calibration 1" was 4.003 and for the "calibration 2" was 4.117. Comparison of the two groups by ANOVA gave a P-value of 0.004 suggesting that there is a significant difference between the two groups.

Both sets of data, presented some indication that the content of chl **a** decreases with increasing growth irradiance. For the purposes of calibration the equation 4.1 was derived from the average values of both calibration tests:

$y = -0.0304x + 5.2889$ (4.1)

where x is the light intensity (μmol $m^{-2}s^{-1}$) and y is the conversion factor between OD_{678} and concentrations of chl **a** (mgl^{-1}) in algal biomass. Linear regression analysis gave R^2=0.901 and a negative slope, as depicted in Figure 4.1.

Table 4.4 Conversion coefficients for chl **a** content (mg l^{-1}) and OD_{678} of *S. subspicatus* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	5.61	0.988	4.44	0.995
15.7	5.71	0.989	4.25	1
31.3	3.55	0.988	4.25	0.999
47.0	3.87	0.989	4.64	0.999
62.7	3.08	0.997	3.62	0.998
78.3	2.19	0.998	3.50	0.984

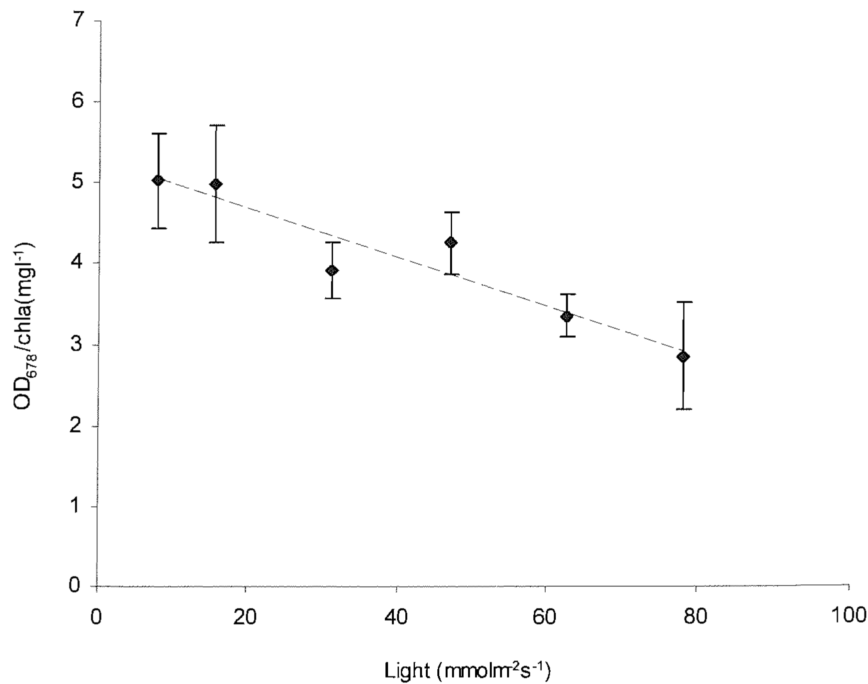


Figure 4.1 Values of *S. subspicatus* cultures for conversion factor from both tests plotted against light intensities. Dashed line is the average between values of two calibration tests. Error bars indicate ± SE.

4.1.3 Cell count

4.1.3.1 C. vulgaris

Conversion coefficients from two independent experiments are given Table 4.5. The value of 32820 derived as an average for both tests was used as the conversion coefficient for further calculations. The average value for the first was 31847 ±1670 and for the second was 33793 ± 1003. Comparison of the two groups by ANOVA gave a P-value of 0.341, which suggests that they are not significantly different.

Table 4.5 Conversion coefficients for cell count (l^{-1}) and OD_{678} of *C. vulgaris* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	26795	0.994	32705	0.996
15.7	34425	0.994	33444	0.997
31.3	32455	0.999	33721	0.996
47.0	31877	0.997	37886	0.966
62.7	28882	0.995	30408	0.988
78.3	30350	0.989	34596	0.985
Average	31847		33793	
± SE	1670		1003	

4.1.3.2 S. subspicatus

Conversion coefficients from two independent experiments are given in Table 4.6. The value of 18851 derived as an average for both tests was therefore used as the conversion coefficient for further calculations. The average value for the first was 17653 ± 1010 and for the second was 20050 ± 682. Comparison of the two groups by ANOVA gave a P-value of 0.077, which suggests that they are not significantly different.

Table 4.6 Conversion coefficients for cell count (l^{-1}) and OD_{678} of *S. subspicatus* cultures derived from calibration experiments, with R^2 values for the original data.

Light (μmol $m^{-2}s^{-1}$)	calibration 1	R^2 for calibration 1	calibration 2	R^2 for calibration 2
7.8	17854	0.983	20150	0.998
15.7	17267	0.947	19080	0.997
31.3	17412	0.996	21369	0.997
47.0	16201	0.968	21375	0.991
62.7	22229	0.983	21154	0.989
78.3	14953	0.982	17173	0.998
Average	17653		20050	
± SE	1010		682	

4.1.4 Discussion

For *C. vulgaris* the relationships between dry weight, chl **a** and cell count with OD_{678} appear to be independent of growth light intensity under the range of conditions tested. Values for conversion coefficients are effectively constant and can be reliably used for data interpretation.

The same is true for *S. subspicatus* with the exception of the relationship between chl**a** and OD_{678}, which appears to depend on the growth light intensity. An equation was obtained for a conversion coefficient but this should be treated with some caution as statistical analysis revealed that only 69% of the variations of data in both experiments are shared.

According to Kirk (1994) some algal cells are able to increase their light harvesting capacity during periods of insufficient light. Experimental results with *Skeletonema costatum* (Riper et al 1956) indicated that chl **a** could be rapidly recycled, with turnover times from 3 to 10 hours. Similar results were reported for *Chlorella pyrenoidosa* (Grumbach et al. 1960). Fogg (1953) points out on existence of two types of response to varying light intensities among algae: those where algae are able to perform chlorophyll turnover and those where this is not possible. The importance

of these findings for the current research is that some algal species (e.g., *S.subspicatus*) have a biochemical capacity to regulate their intracellular pigment components on a time scale of several hours, that is, within less than a generation time. Thus, the photosynthetic capacity of the cells may be affected by the amount of the main pigment present.

Values of the conversion coefficients used in subsequent work for all parameters and both species are summarised in Table 4.7.

Table 4.7 Empirically established coefficients OD_{678} *vs.* biomass dry weight, concentration of chlorophyll a and, cell amount (cells l^{-1}) obtained for *S. subspicatus* and *C. vulgaris*.

Coefficient	*C. vulgaris*	*S. subspicatus*
OD_{678} *vs.* dry weight (mg l^{-1})	269.0	350.1
OD_{678} *vs.* chl a (mg l^{-1})	2.25	$y = -0.0304\,x + 5.2889$
OD_{678} *vs.* cell l^{-1}	32820	18851

4.2 Effects of carbon enrichment on algal growth and activity

Growth characteristics, oxygen production and nutrient uptake were assayed at bicarbonate concentrations ranging from 0.09 to 18.49 mmol l^{-1} by using batch cultures of *S. subspicatus* and *C. vulgaris*.

4.2.1 Growth

Figure 4.2 and Figure 4.3 show the growth response curves (exponential phase and onset of stationary phase). Rates of growth during the exponential phase of both *S. subspicatus* and *C. vulgaris* cultures exhibited a clear response to changes in carbon concentration (Table 4.8).

Table 4.8 Average values (n=3) of specific growth rates (μ) for *S. subspicatus* and *C.vulgaris* cultures grown with different concentrations of bicarbonate in the medium.

Carbon (mmol l^{-1})	μ (h^{-1})	
	S.subspicatus	*C.vulgaris*
0.92	0.026	0.013
1.85	0.036	0.038
2.77	0.043	0.045
3.70	0.047	0.046
5.55	0.048	0.056
7.40	-	0.061
9.25	0.058	0.064
11.10	0.052	0.065
12.94	0.062	0.063
14.79	0.063	0.066
16.64	0.063	0.063
18.49	0.061	0.062

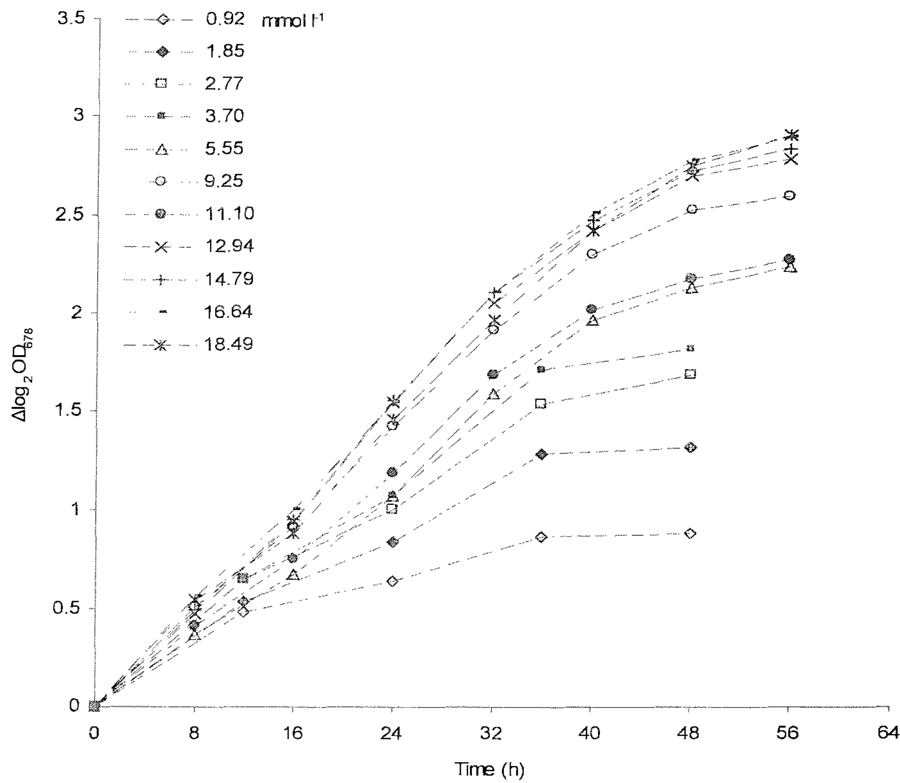


Figure 4.2 Growth responses expressed as Δlog_2OD_{678} for *S. subspicatus* cultures grown with different concentrations of carbon source (HCO_3^-) in the medium.

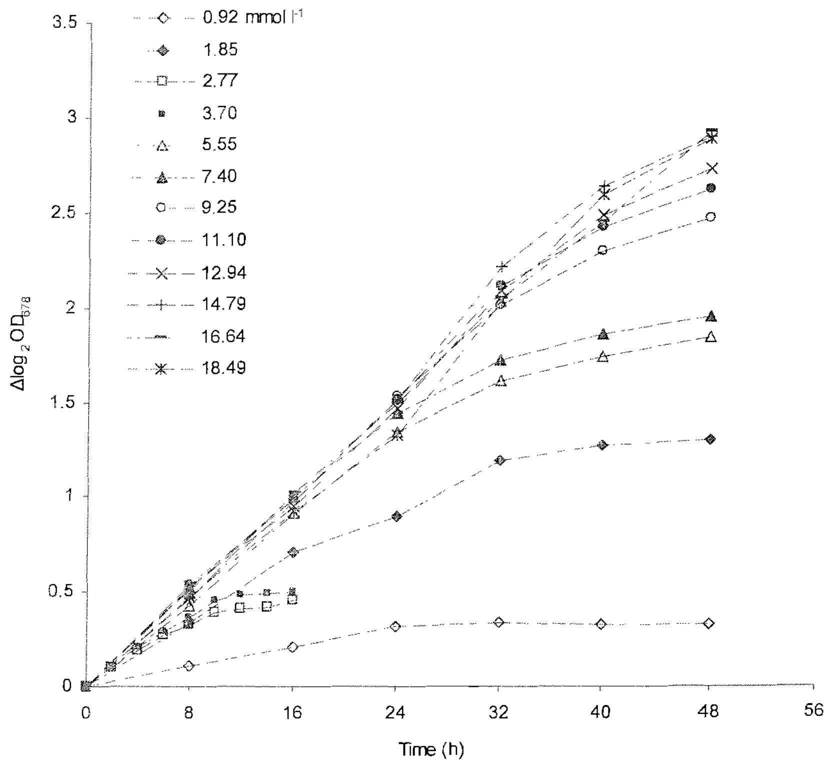
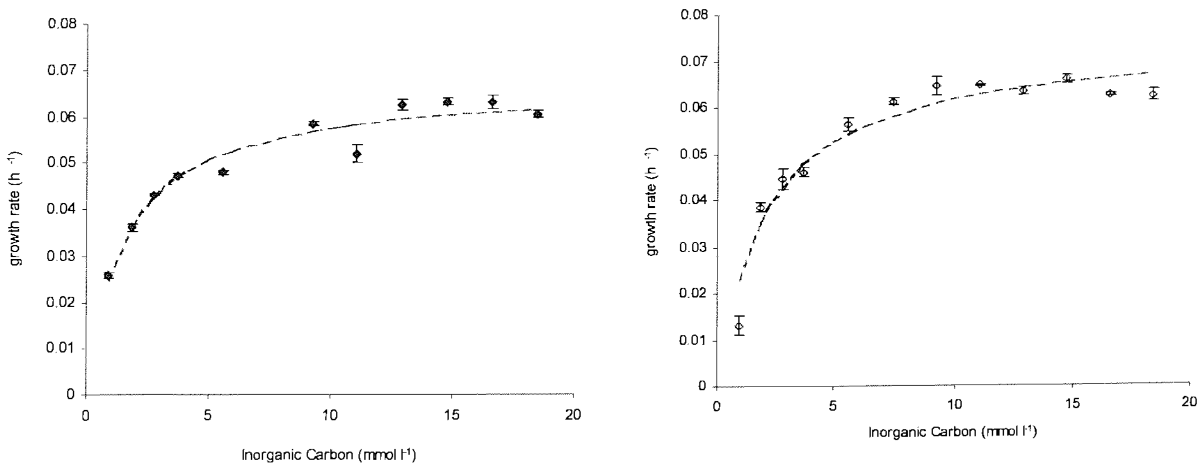


Figure 4.3 Growth responses expressed as Δlog_2OD_{678} for *C. vulgaris* cultures grown with different concentrations of carbon source (HCO_3^-) in the medium.

Enrichment of the experimental medium with bicarbonate stimulated specific growth rates of *S. subspicatus* until the nutrient concentration reached 9.25 mmol C l^{-1}; from 9.25 mmol C l^{-1} to 18.49 mmol C l^{-1} the rates were 0.060 ± 0.002 (±SE) h^{-1}.

C. vulgaris cultures exhibited a rise in specific growth rate from 0.013 to 0.061 h^{-1} average (n=3) until the concentration of inorganic carbon in the medium amounted to 7.40 mmol C l^{-1}; the specific growth rates remained at 0.063 ± 0.001 (±SE) h^{-1} until 18.49 mmol C l^{-1}.

Under the conditions of light and temperature used in the experiment the maximum carbon-specific growth rates μ_{max} calculated using the Monod equation were 0.067 h^{-1} for *S. subspicatus*, and 0.075 h^{-1} for *C. vulgaris*. The response of algal specific growth rates to the concentration of residual inorganic carbon was also described by the Monod equation leading to K_s values of 1.59 mmol C l^{-1} for *S. subspicatus* and 2.11 mmol C l^{-1} for *C. vulgaris*. Figure 4.4 shows the experimental data plotted against the line generated using the Monod equation with these parameters.



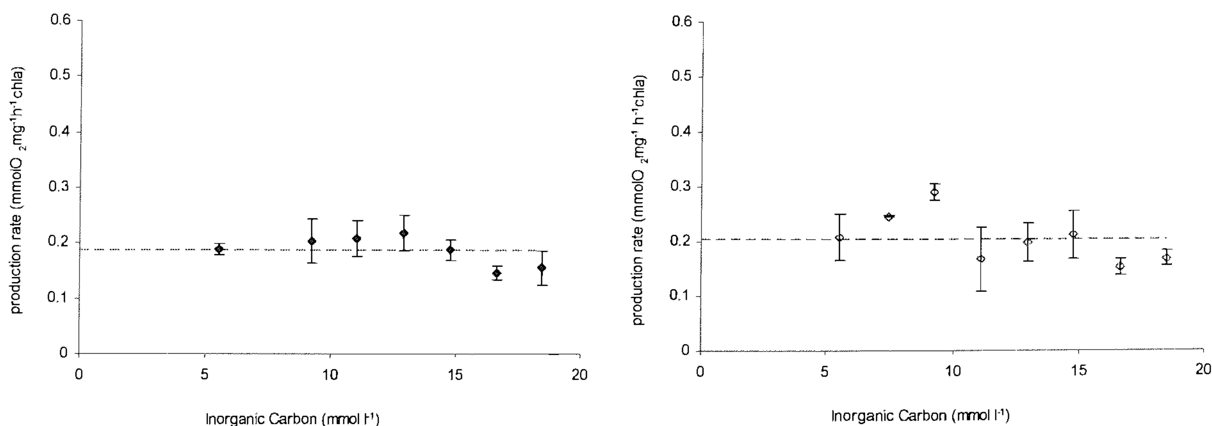
a) *S. subspicatus* b) *C. vulgaris*

Figure 4.4 Effect of inorganic carbon concentration on the specific growth rates of *S.subspicatus* (a) and *C. vulgaris* (b) Dashed lines represent the Monod fitting to the experimental results. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

The correlation coefficient between experimental and calculated values is 0.944 for *S.subspicatus* and 0.931 for *C. vulgaris*, indicating that growth limitation with respect to bicarbonate shows a normal substrate limited response. The minimum bicarbonate concentration required for non-limited growth by both species appears to be around 10-13 mmol C l^{-1}.

4.2.2 Photosynthetic oxygen production

Rates of photosynthetic oxygen production were measured for both species: the results are shown in Table 4.9 and Figure 4.5. *S. subspicatus* showed a range from 0.147 to 0.219 mmol O_2 $mg^{-1}h^{-1}$chl a, while *C. vulgaris* ranged from 0.153 to 0.290 mmol $O_2mg^{-1}h^{-1}$chl a.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.5 Effect of inorganic carbon concentration on the rate of photosynthetic oxygen production by *S. subspicatus* (a) and *C. vulgaris* (b). Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

Taking into account the high values of deviation between replicates (13.5% on average), there appears to be no discernible difference in the rate of photosynthetic oxygen production at different initial substrate carbon concentrations.

Table 4.9 Average rates (n=3) of photosynthetic oxygen production by *S. subspicatus* and *C.vulgaris* cultures grown with different concentrations of inorganic carbon in medium.

Carbon	Photosynthetic oxygen production (mmol O_2 $mg^{-1}h^{-1}$ chl a)	
(mmol l^{-1})	*S. subspicatus*	*C. vulgaris*
5.55	0.190	0.207
7.40	-	0.246
9.25	0.204	0.290
11.10	0.208	0.167
12.94	0.219	0.197
14.79	0.188	0.212
16.64	0.147	0.153
18.49	0.155	0.168

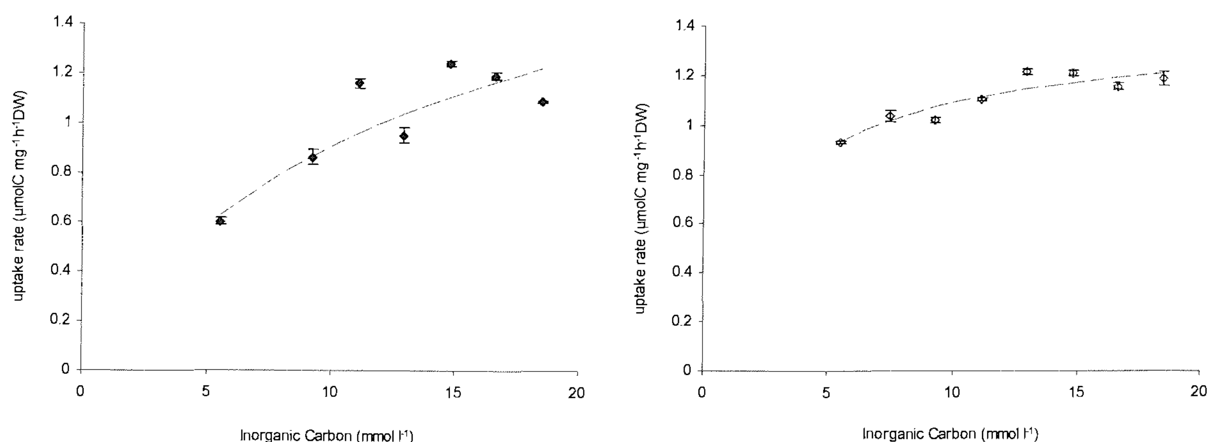
4.2.3 Inorganic carbon uptake

Inorganic carbon uptake was measured by depletion of carbon source in the medium. The results are shown in Table 4.10 and Figure 4.6.

Uptake ranged from 0.603 to 1.238 μmol C $mg^{-1}h^{-1}$DW for *S. subspicatus* and from 0.934 to 1.217 μmolC$mg^{-1}h^{-1}$DW for *C. vulgaris*. Although there is some variability in the individual data points, there is a clear upward trend in uptake rate with increasing initial substrate concentrations for both species. The rates of uptake by *S. subspicatus* appear higher than those for *C. vulgaris*. This is supported by calculation of the uptake rates using the Michaelis-Menten equation, shown as dashed lines in Figure 4.6. The correlation coefficients between experimental and calculated values for *S. subspicatus* and *C.vulgaris* were 0.658 and 0.796, respectively.

Use of the Michaelis-Menten equation gave maximum uptake rates, V_{max}, for *S.subspicatus* and C. *vulgaris* of 2.05 and 1.38 μmol C $mg^{-1}h^{-1}$ DW, respectively. The value of K_m for *S. subspicatus* was 12.55 mmol C l^{-1}, which is considerably higher

than that of 2.63 mmol C l^{-1} for *C. vulgaris*. Correlation coefficients indicate an insufficient fit to the model.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.6 Rates of inorganic carbon uptake by *S. subspicatus* (a) and *C. vulgaris* (b) grown with different initial concentrations of inorganic carbon. Dashed lines represent the Michaelis-Menten equation fitting to the experimental results. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

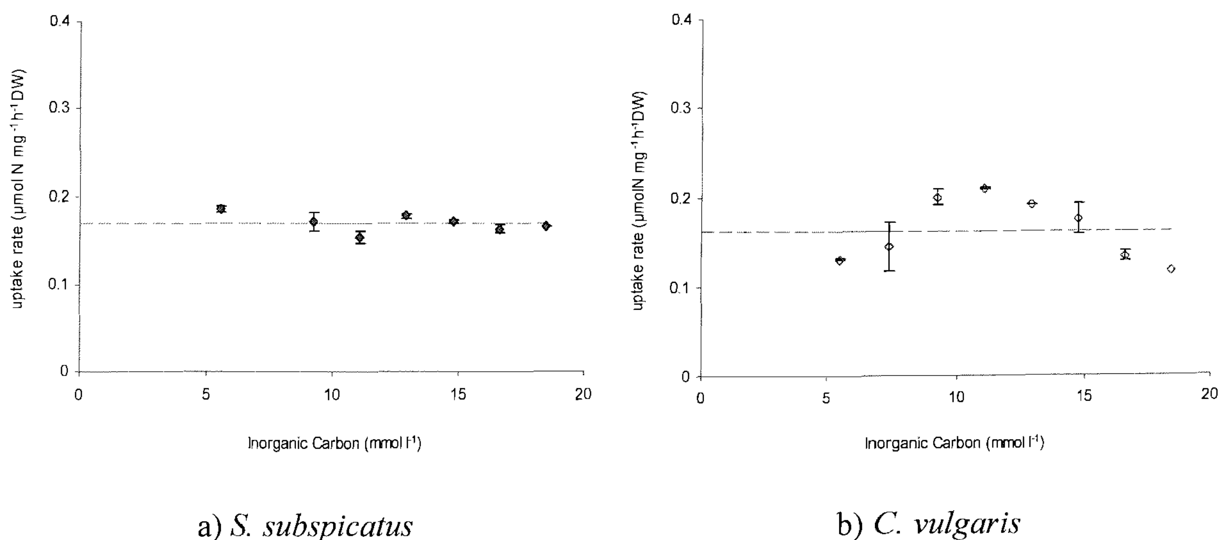
Table 4.10 Average rates (n=3) of inorganic carbon uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic carbon in the medium.

Carbon (mmol l^{-1})	Rates of inorganic carbon uptake (µmol C $mg^{-1}h^{-1}$DW)	
	S. subspicatus	*C. vulgaris*
5.55	0.603	0.934
7.40	-	1.039
9.25	0.864	1.020
11.10	1.160	1.106
12.94	0.951	1.217
14.79	1.238	1.208
16.64	1.191	1.157
18.49	1.086	1.187

The carbon concentrations in the control flasks without algae remained in the range of initially added carbon (± 1.5%) throughout sampling time, and showed no significant differences in carbon concentration between the first and the last measurements indicating that the observed uptake in the main experiments was due to the algae.

4.2.4 Nitrate uptake

Nitrate uptake was measured by depletion from the medium. The results are shown in Figure 4.7. Uptake rates for *S. subspicatus* remained fairly constant irrespective of the inorganic carbon content of the medium, with an average value of 0.170 ± 0.004 (±SE) μmol N $mg^{-1}h^{-1}$DW. Uptake rates by *C. vulgaris* also showed no apparent variation with increasing concentrations of inorganic carbon substrate, and an average value of 0.163 ± 0.012 (±SE) μmol N $mg^{-1}h^{-1}$DW (the values of rates of nitrogen uptake by each of the species are given in Appendix C, Table C.1).

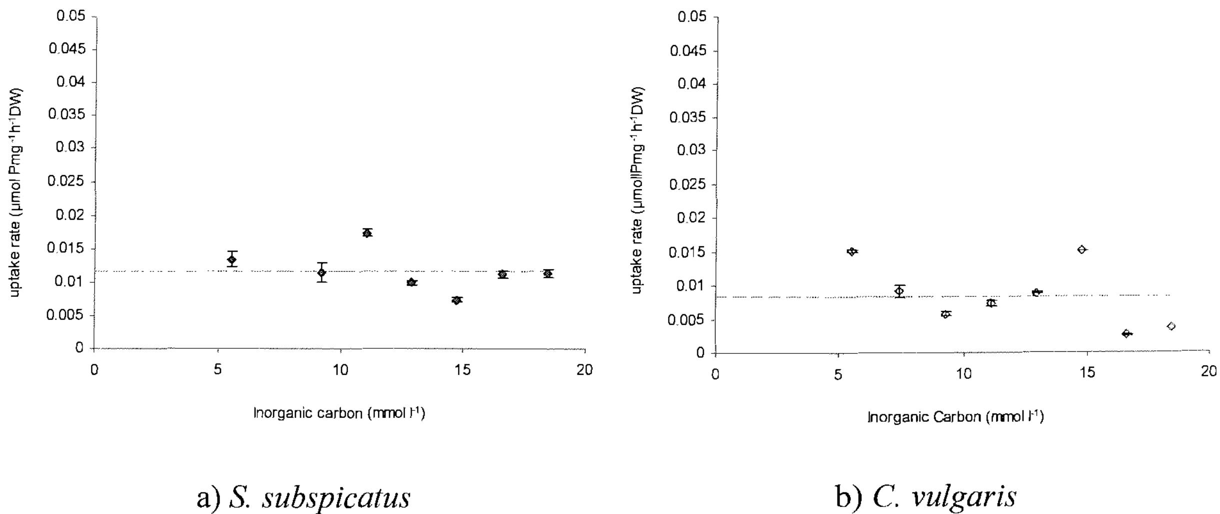


a) *S. subspicatus* b) *C. vulgaris*

Figure 4.7 Rates of nitrate uptake by *S. subspicatus* (a) and *C. vulgaris* (b) with grown different initial concentrations of inorganic carbon. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.2.5 Phosphate uptake

Residual inorganic phosphate concentrations were measured by depletion of the anion from the medium. The results are shown in Figure 4.8. For *S. subspicatus* the rates of phosphate uptake detected were between 0.0073 and 0.0172 with the average value of 0.011 ± 0.001 (±SE) μmol P $mg^{-1}h^{-1}$DW. For *C. vulgaris* the rates were between 0.0026 and 0.0152 with the average value of 0.009± 0.002 (±SE) μmol P $mg^{-1}h^{-1}$DW (the values of rates of phosphate uptake by each of the species are given in Appendix C, Table C.2). Due to low concentrations it is impossible to draw any firm conclusions concerning the kinetics of the nutrient uptake.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.8 Rates of phosphate uptake by *S. subspicatus* (a) and *C. vulgaris* (b) grown with different initial concentrations of inorganic carbon Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.2.6 pH

Variation in onset times of the stationary phase for algal cultures grown with different concentrations of carbon in the medium may have been influenced by rising pH. As shown in Table 4.11, terminal pH was in the range 11 - 11.5 for *S. subspicatus* suspensions and 10.1 - 10.9 for *C. vulgaris*.

4.2.7 Experiments to investigate the effects of pH on growth rates

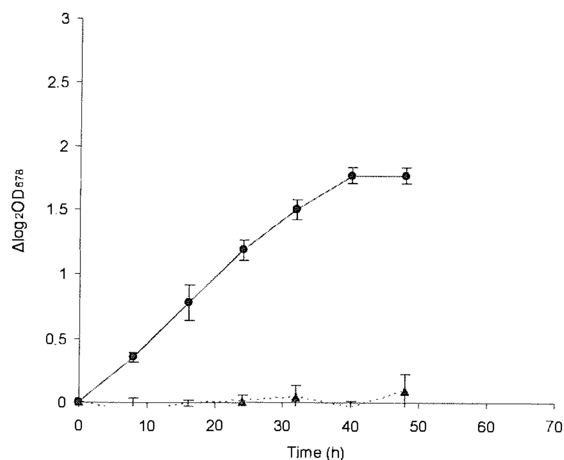
Table 4.12 shows the growth rate, onset of stationary phase and final pH for *S.subspicatus* and *C. vulgaris* cultures grown in open (variant 1) and closed (variant 2) flasks. Growth response curves for *C. vulgaris* grown with different concentrations of carbon in the medium generally showed a similar pattern to *S. subspicatus.*

Table 4.11 pH values of the medium at the beginning and the end of carbon enrichment experiments.

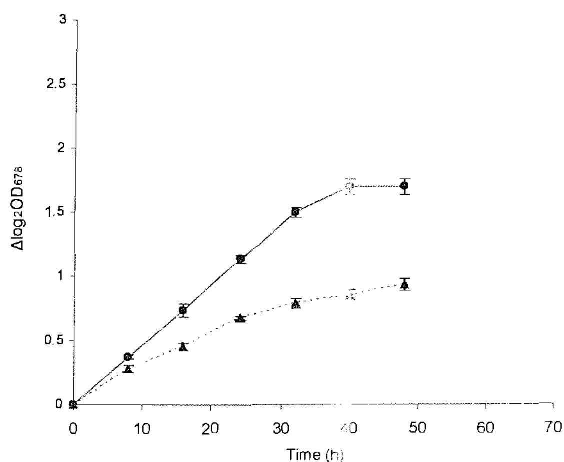
Carbon (mmol l^{-1})	Initial pH	Final pH	
		S.subspicatus	*C. vulgaris*
0.92	7.2	11.0	10.1
1.85	7.4	11.0	10.2
2.77	7.6	11.0	10.5
3.70	7.7	11.1	10.6
5.55	7.8	11.1	10.7
7.40	7.8	-	10.7
9.25	7.9	11.3	10.8
11.10	7.9	11.3	10.8
12.94	7.8	11.3	10.8
14.79	7.8	11.4	10.9
16.64	7.8	11.4	10.9
18.49	7.9	11.5	10.8

At 0.18 mmol Cl^{-1} there was a significant impact on the growth rate and the duration of the exponential phase of both species: the growth in the closed flasks (variant 2) appeared to be nearly inhibited (Figure 4.9 (a) and Figure 4.104.10 (a)). Although for *C.vulgaris* cultures there appeared to be some early growth followed by a clearing (as shown by decreasing in OD_{678}) of the culture possibly indicating cell lysis. At 1.85 mmol Cl^{-1} both species showed a progress in growth, however, considerably different between the variants (Figure 4.9(b) and Figure 4.10 (b)). At a higher

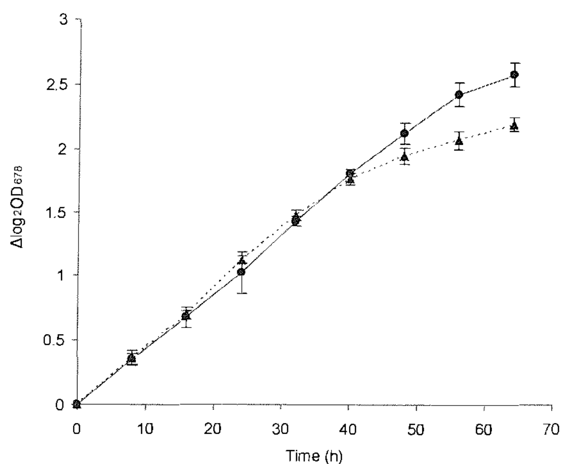
concentration of 9.25 mmol C l^{-1} the growth rate was similar but the onset of the stationary phase was approximately 8-10 hours earlier (Figure 4.9 (c) and Figure 4.10 (c)).



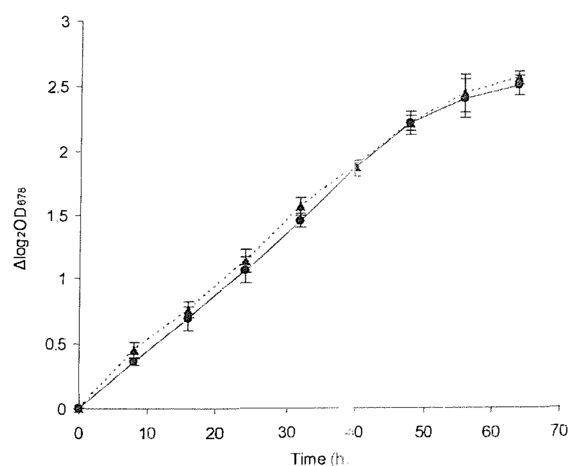
(a) 0.18 mmol C l^{-1}



(b) 1.85 mmol C l^{-1}



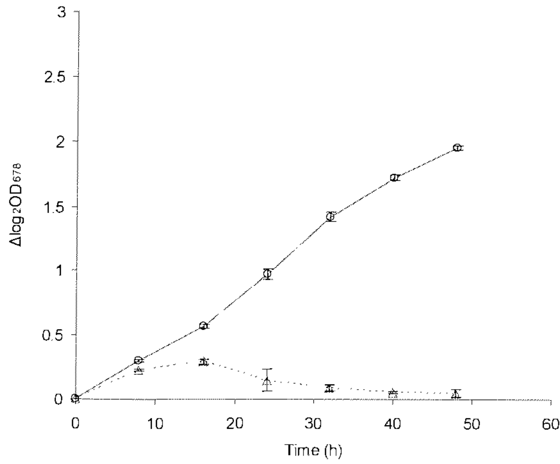
(c) 9.25 mmol C l^{-1}



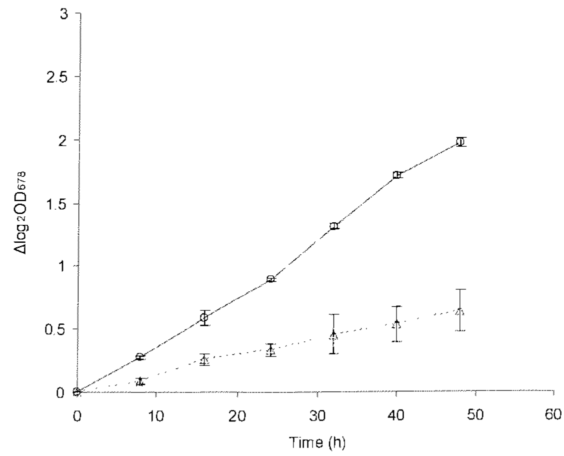
(d) 18.49 mmol C l^{-1}

Figure 4.9 Growth response curves for *S. subspicatus* cultures grown in open (●) and closed (▲) flasks at different concentrations of carbon source (HCO_3^-) in the medium. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

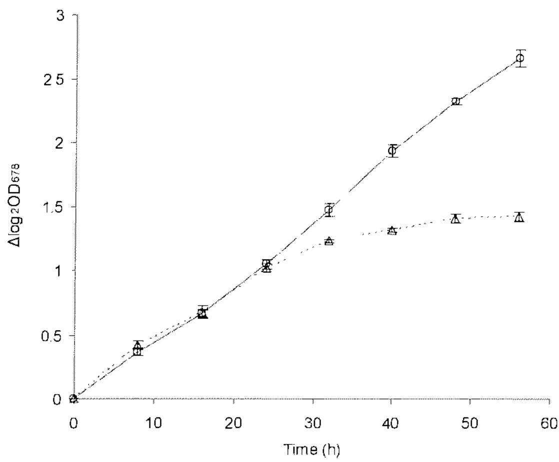
There was no apparent difference between the two cultures in either growth rate or onset of stationary phase at higher values of initial substrate concentration (18.49 mmol C l^{-1}) (Figure 4.9(d) and Figure 4.10(d)).



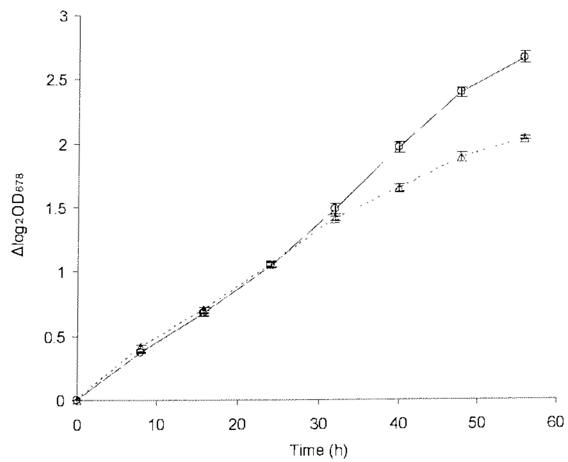
(a) 0.18 mmol C l^{-1}



(b) 1.85 mmol C l^{-1}



(c) 9.25 mmol C l^{-1}



(d) 18.49 mmol C l^{-1}

Figure 4.10 Growth response curves for *C. vulgaris* cultures grown in open (O) and closed (△) flasks at different concentrations of carbon source (HCO_3^-) in the medium. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

Figure 4.11 and Figure 4.12 show time course progress in pH values of the experimental suspensions. The considerable rise in pH occurred at first 8 hours in all cultures (except experimental with *C.vulgaris* with 0.18 mmol Cl^{-1} in the medium) followed by a graduate increase towards end of the exponential phase of the growth.

The results show that for both species atmospheric carbon dioxide had a significant influence on the growth response, with the effect being most pronounced at low initial carbon concentrations. The final pH value for each experiment was similar in both open and closed flasks and showed a graduate rise in values (except first 8 h), which may indicate that pH is not the main factor influencing these differences.

Table 4.12 Average values (n=3) of specific growth rates (μ), onset of stationary phase, and pH values for *S. subspicatus* and *C. vulgaris* cultures grown in open and closed flasks.

Carbon (mmol l^{-1})	*S. subspicatus*	μ (h^{-1})	Onset of stationary phase (h)	pH
0.18	open flasks	0.048	40	10.7
	closed flasks	-	-	-
1.85	open flasks	0.047	40	11.0
	closed flasks	0.029	24	11.1
9.25	open flasks	0.044	56	11.0
	closed flasks	0.045	40	11.0
18.49	open flasks	0.046	56	10.2
	closed flasks	0.047	56	10.6
Carbon (mmol l^{-1})	***C. vulgaris***	**μ (h^{-1})**	**Onset of stationary phase (h)**	**pH**
0.18	open flasks	0.042	40	8.9
	closed flasks	0.028	8	8.9
1.85	open flasks	0.041	40	9.5
	closed flasks	0.014	32	10.0
9.25	open flasks	0.047	40	9.9
	closed flasks	0.043	24	9.8
18.49	open flasks	0.048	40	9.9
	closed flasks	0.044	40	9.7

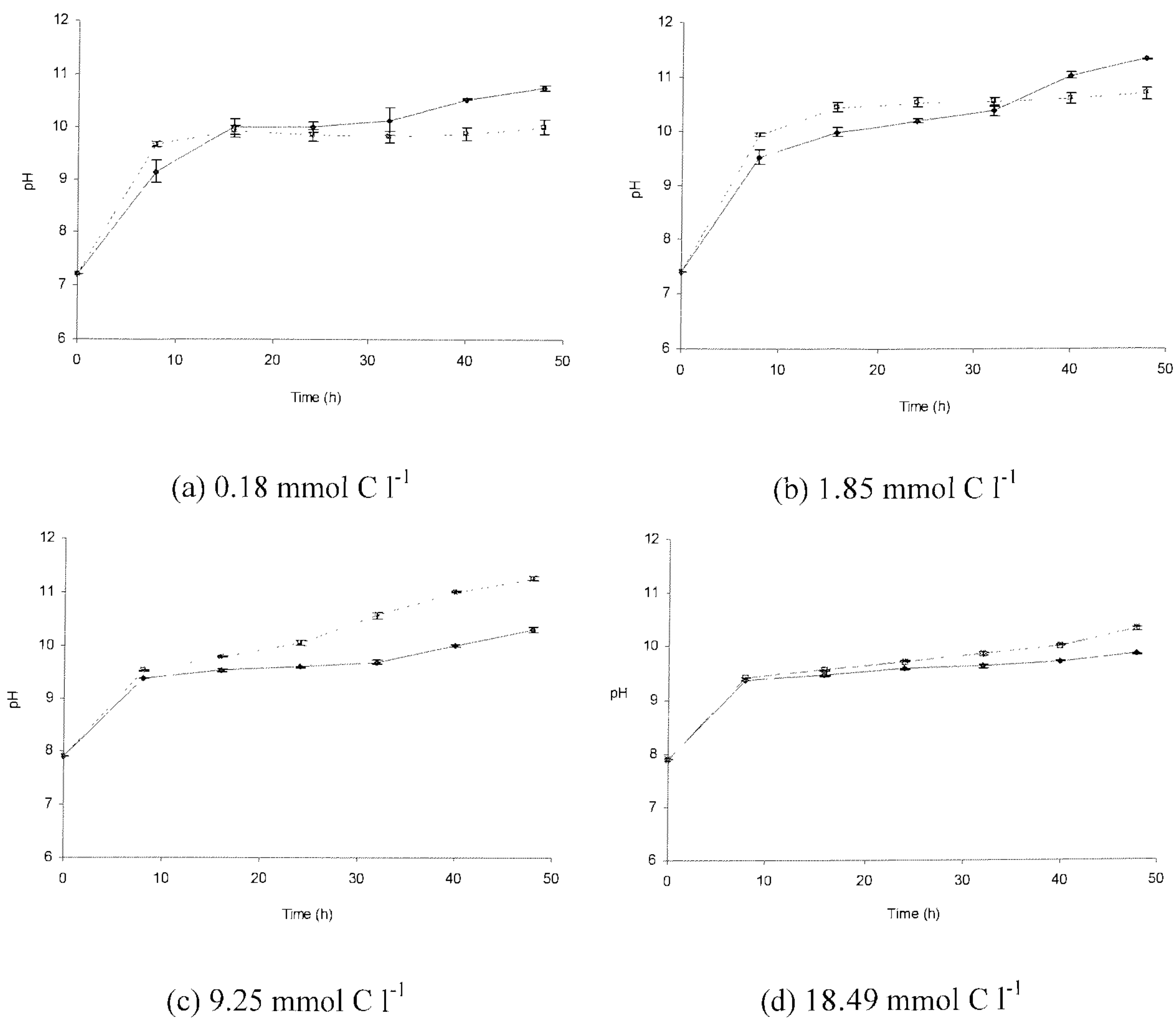
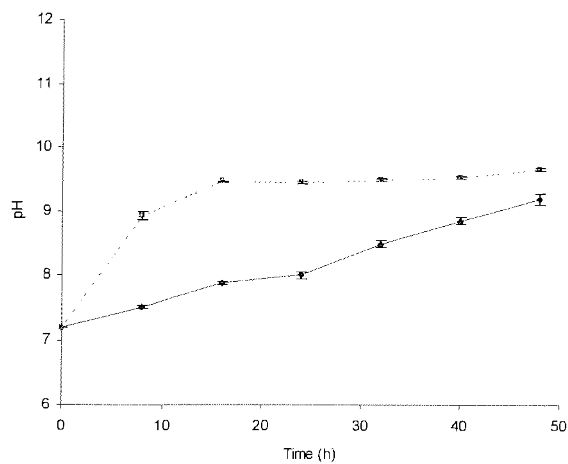
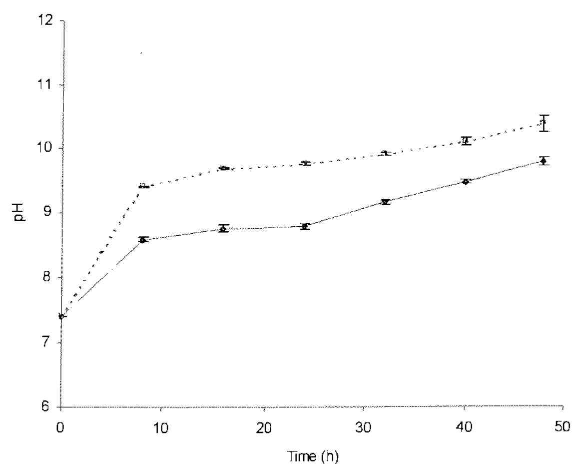


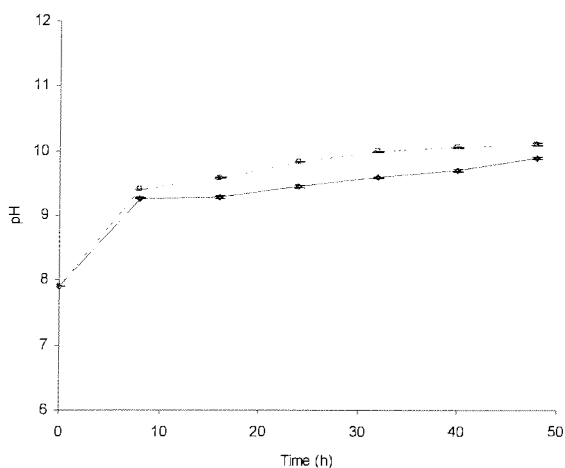
Figure 4.11 Changes in pH values for *S.subspicatus* cultures grown in open (♦) and closed (□) flasks at different concentrations of carbon source (HCO_3^-) in the medium. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.



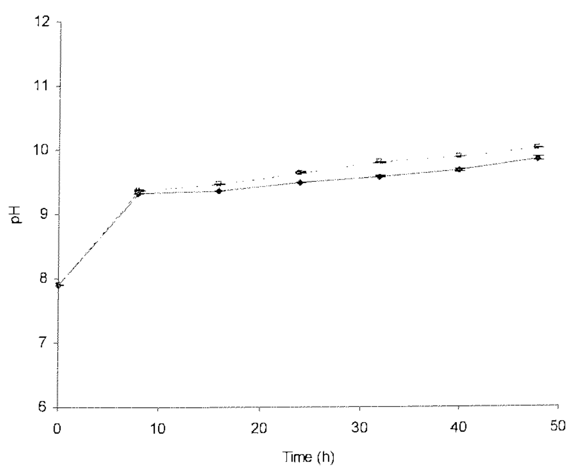
(a) 0.18 mmol C l^{-1}



(b) 1.85 mmol C l^{-1}



(c) 9.25 mmol C l^{-1}



(d) 18.49 mmol C l^{-1}

Figure 4.12 Changes in pH values for *C. vulgaris* cultures grown in open (♦) and closed (□) flasks at different concentrations of carbon source (HCO_3^-) in the medium. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.2.8 Discussion

S. subspicatus had μ_{max} of 0.067 h^{-1} and *C. vulgaris* had μ_{max} of 0.075 h^{-1}, however, the highest observed values of specific growth rates were 0.063 h^{-1} for *S.subspicatus* and 0.066 h^{-1} for *C. vulgaris* cultures at an initial concentration of 14.79 mmol C l^{-1} in the medium. For comparative purposes, maximum growth rates found by other researchers for *Chlorella* species under various conditions are given in Appendix A.

The ability of *C. vulgaris* and *S. subspicatus* to grow at maximum rates in batch culture at inorganic carbon concentrations as low as 14.79 mmol C l^{-1} appears to be a common characteristic of many freshwater and marine algae (Goldman & Toerien 1972; Goldman & Graham 1981; Novak & Brune 1985; Hecky *et al.* 1993; Qiu & Gao 2002) and is an indication of the high affinity for inorganic carbon. Thielmann *et al.* (1989) presented evidence indicating that *Scenedesmus* is able to utilise both undissociated carbon dioxide and bicarbonate ions, only 10 to 20 μmol l^{-1} being required for maximum growth whereas the corresponding value for undissociated carbon dioxide is 80 μmol $l^{-1.}$ Contrary to the findings of (Tsuzuki *et al.* 1985), the current research clearly indicated that bicarbonate is a suitable carbon source for *C.vulgaris*.

There is some evidence that at 100% CO_2 concentrations (Sorokin 1962) or high concentrations of bicarbonate (Osterlind 1949) there may be toxicity or reduction in growth rates. The reasons for this are unclear but could be due to a requirement for O_2 at the cell surface or increasing pH values. In the present work no evidence was found of growth inhibition at the highest concentration of bicarbonate used of 18.49 mmol C l^{-1}.

There has been some debate in the past as to the relative availability of different forms of carbon from CO_2 to HCO_3^- and CO_3^{2-} (Goldman *et al.* 1974). Kern (1960) noted that the conversion of bicarbonate to CO_2 at pH values above 10 is relatively slow. Under the conditions used it can therefore be assumed that carbon availability will not cause any rate limitation detrimental to achieving a maximum specific growth rate.

According to Gavis and Ferguson (1975) the uptake parameters for carbon dioxide, V_{max} and K_m (from the Michaelis-Menten equation) are influenced by pH. The conclusion drawn from their experimental results was that organisms with large values of the ratio K_m/V_{max} assimilated CO_2 slowly at low CO_2 concentrations, at any pH and when other nutrients were available at nonlimiting concentrations. Organisms with small values of K_m/V_{max}, however, could assimilate CO_2 rapidly at low concentrations. *S. subspicatus* showed K_m of 12.55 mmol C l^{-1} at V_{max} of 2.05 μmol C $mg^{-1}h^{-1}$DW, giving a ratio of 6.12. *C. vulgaris* exhibited K_m of 15.30 mmol C l^{-1} at V_{max} of 1.38 μmol C $mg^{-1}h^{-1}$DW, thus producing a K_m/V_{max} ratio of 11.01. It is evident that *C. vulgaris* had lower rates of CO_2 assimilation than

S. subspicatus, and as a consequence the CO_2 uptake rate for the latter was transport limited.

The use of the Monod equation to model the results is further supported by Goldman *et al.* (1974) who showed that the relationship between C-specific growth rate and the concentration of dissolved inorganic carbon in a medium including all carbon species was described well by the Monod equation for *C. vulgaris* and reasonably well for *Scenedesmus obliquus*.

In the concentration range used in the carbon-specific growth experiments there is clear evidence of carbon limitation at bicarbonate concentrations below 14.79 mmol C l^{-1}. Above this concentration growth rates remain constant but the maximum growth yield does not increase further with increasing inorganic carbon concentration in the medium. The growth of phytoplankton populations in a water body is accompanied by a decrease in the ambient concentration of total inorganic carbon (DIC), which may fall to micromolar concentrations. Low concentrations of inorganic carbon in the aquatic environment with actively growing algae may be maintained for long periods of time due to the low rate of CO_2 diffusion from air. The decrease in DIC concentrations is accompanied by an increase in pH values, which may rise above 10, and also by an increase in dissolved O_2 to levels of 100 to 200% of the air-equilibrium value (Goldman & Toerien 1972; Azov 1982; Nimer *et al.* 1997; Menendez *et al.* 2001). Thus, carbon limitation might develop in intense algal blooms even in water bodies with high DIC concentrations. In these experiments the maximum algal yield is probably limited not by the available carbon but by the development of unfavourable conditions in the culture medium causing the dense cultures to enter the stationary phase, or by cell membrane or surface specific reactions (Arad *et al.* 1980).

In more recent work it has been postulated that unicellular algae respond to limitations in extracellular dissolved inorganic carbon (DIC) by the induction of a high DIC-affinity photosynthesis, described as a carbon concentrating mechanism (CCM). This results from the induction of active CO_2 and/or active HCO_3^- transport systems and, in some microalgae, the induction of an extracellular CA activity (Badger *et al.* 1980; Matsuda & Colman 1995; Matsuda *et al.* 1998; Moroney & Somanchi 1999). The signal for CCM induction may be also provided by the change in the CO_2/O_2

concentration ratio in the external medium, since more substrate will be available for the oxygenase activity of Rubisco, and hence there will be an increase in phosphoglycolate and other photorespiratory pathway intermediates (Marcus *et al.* 1983). As shown by Bozzo *et al.* (2000) there is no difference in the maximum rate of photosynthesis for *Chlorella kessleri* cultured at different concentrations of CO_2 at the present of HCO_3^-. The results were similar to those for other algal groups (Badger *et al.* 1980; Mayo *et al.* 1986; Matsuda & Colman 1995) and to cases with two *Chlorella* mutants (Matsuda & Colman 1995; Matsuda & Colman 1995).

4.3 Effects of nitrogen enrichment on algal growth and activity

Growth characteristics and nutrient uptake were assayed at nitrate concentrations (NO_3^-) ranging from 0 to 4.56 mmol l^{-1} for *S. subspicatus* and *C. vulgaris*.

4.3.1 Growth

Growth of batch cultures produced exponential growth rates as shown in Table 4.13. Nitrate-dependent growth rates for both species reached a maximum value at concentrations above 0.11 mmol N l^{-1} (Figure 4.13). In the absence of NO_3^- in the medium, cultures of *S. subspicatus* grew at a rate of 0.035 h^{-1} and *C. vulgaris* at 0.051 h^{-1}. The relationship between N-specific growth rates and the presence of particular concentrations of nitrate in the growth medium fits the Monod model, suggesting that nitrogen is a limiting factor for algal growth. Calculation of the N-dependent maximum growth rate μ_{max} and the associated half saturation constant K_s for cultures of *S. subspicatus* gave values of 0.048 h^{-1} and 0.013 mmol N l^{-1}, while for *C. vulgaris* μ_{max} was 0.060 h^{-1} and K_s 0.012 mmol N l^{-1}. The low value of K_s indicates that both species can attain maximum growth rates at very low concentrations of nitrate. Correlation coefficients for the experimental and calculated N-specific growth rates gave R^2 = 0.71 for *S. subspicatus* and 0.81 for *C. vulgaris*.

a) *S. subspicatus* b) *C. vulgaris*

Figure 4.13 Effect of inorganic nitrogen concentration on the specific growth rates of *S. subspicatus* (a) and *C. vulgaris* (b) cultures Dashed lines represent the Monod fitting to the experimental results. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

Table 4.13 Average values (n=3) of specific growth rates (μ), for *S. subspicatus* and *C.vulgaris* cultures grown with different concentrations of nitrate in the medium.

Nitrate (mmol l^{-1})	μ (h^{-1})	
	S. subspicatus	*C.vulgaris*
0	0.035	0.027
0.06	0.038	0.044
0.11	0.045	0.061
0.23	0.045	0.058
0.46	0.046	0.064
0.91	0.048	0.062
1.14	0.047	0.055
2.28	0.047	0.059
3.42	0.049	0.055
4.56	0.044	0.056

4.3.2 Inorganic carbon uptake

Rates of inorganic carbon uptake by cultures grown with different initial concentrations of nitrate in the medium are given in Appendix C (Table C.3) and range from 0.384 to 0.664 μmol C mg $^{-1}h^{-1}$DW for *S. subspicatus* and 0.466 to 1.985 μmol N $mg^{-1}h^{-1}$DW for *C. vulgaris* on average (n=3). Figure 4.14 shows that for *S. subspicatus* the uptake rate appears initially to be dependent on the nitrate concentration but above 0.9 mmol N l^{-1} it remains relatively constant at around 0.554 ± 0.040 (±SE) μmol C $mg^{-1}h^{-1}$DW. For *C. vulgaris*, the dependence between inorganic carbon uptake and the concentration of nitrate in the growth medium was not clearly shown, but gave an average value of 0.889 ±0.143 (±SE) μmol C mg $^{-1}h^{-1}$ DW within the tested concentration range.

The results from *S. subspicatus* could be modelled using the Michaelis-Menten equation (Figure 4.14a). The value of V_{max} calculated for *S. subspicatus* from the empirical data was 0.54 μmol C $mg^{-1}h^{-1}$DW with a half saturation concentration K_m of

0.02 mmol N l^{-1}. The correlation coefficient for calculated and experimental values was $R^2 = 0.68$ for *S.subspicatus*. The low R^2 values indicate the limited suitability of the model, probably due to the ability of the algae to support the growth with internal reserves of nitrogen at the absence of extracellular source of the nutrient.

a) *S. subspicatus* b) *C. vulgaris*

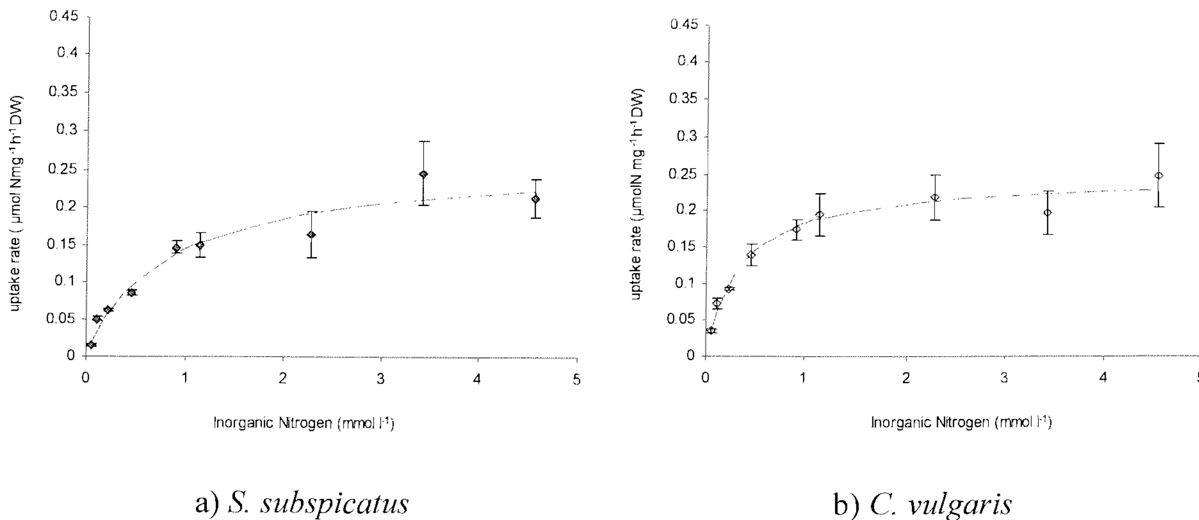
Figure 4.14 Rates of inorganic carbon uptake by *S. subspicatus* (a) and *C. vulgaris* (b) cultures grown with different initial concentrations of inorganic nitrogen Dashed lines represent (a) the Michaelis-Menten fitting, and (b) the average values of carbon uptake rates. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.3.3 Nitrate uptake

Average rates (n=3) of nitrate uptake by *S. subspicatus* and *C. vulgaris* cultures are shown in Appendix C (Table C.4). Values for *S. subspicatus* were between 0.015 to 0.245 μmol N $mg^{-1}h^{-1}$DW at 0.06 to 3.42 mmol N l^{-1}. Uptake rates for *C. vulgaris* gradually increased from 0.035 to 0.247 μmol N $mg^{-1}h^{-1}$DW between 0.06 and 4.56 mmol N l^{-1} in the medium.

The rate of uptake and the concentration of nitrate in the growth medium can be modelled by a hyperbolic relationship for both species. Figure 4.15 shows the rates of nitrogen uptake by *S. subspicatus* and *C. vulgaris* with dashed lines representing calculated values using the Michaelis-Menten equation. V_{max} and K_m for nitrate uptake by *S. subspicatus* were 0.261 μmol N $mg^{-1}h^{-1}$DW and 0.803 mmol N l^{-1}, respectively. *C. vulgaris* cultures showed a V_{max} value of 0.245 μmol N $mg^{-1}h^{-1}$DW and K_m of 0.336 mmol N l^{-1}. Although there were obvious differences in values of half

saturation constants, responsible for the shapes of the curves, there is obvious similarity in V_{max} values for both species. Correlation coefficients between experimental and calculated values were $R^2 = 0.97$ for *S. subspicatus* and 0.98 for *C. vulgaris* with P-value < 0.0001 for both species.



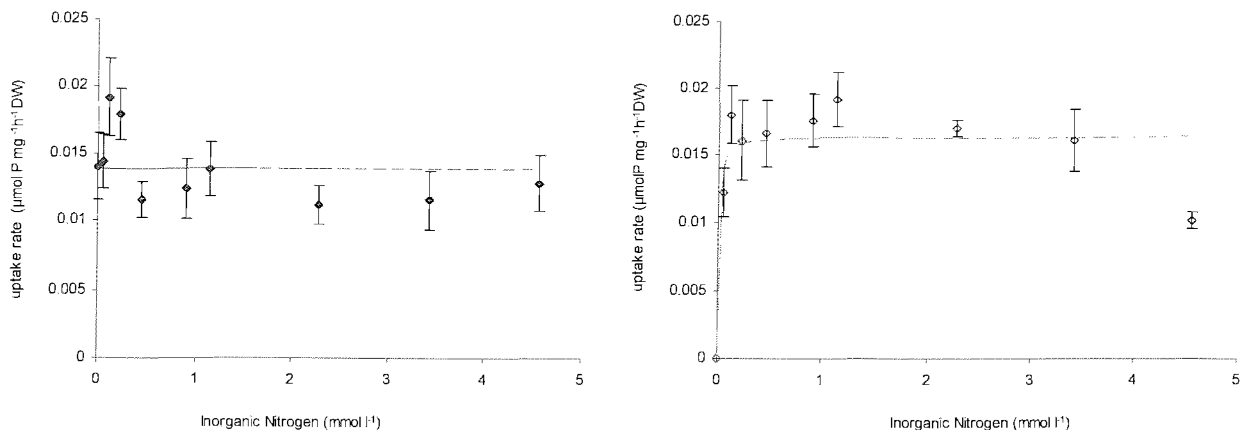
a) *S. subspicatus* b) *C. vulgaris*

Figure 4.15 Rates of nitrate uptake by *S. subspicatus* (a) and *C. vulgaris* (b) cultures grown with different initial concentrations of inorganic nitrogen Dashed lines represent the Michaelis-Menten fitting to the experimental results. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.3.4 Inorganic phosphorus uptake

Uptake of phosphate by *S. subspicatus* remained relatively constant despite differences in concentrations of inorganic nitrogen in growth media, and range between 0.011 and 0.019 with the average rate of 0.014 ± 0.001 (±SE) μmol P $mg^{-1}h^{-1}$ DW. Uptake by *C. vulgaris* increased rapidly with increasing nitrogen concentration to 0.016 ± 0.001 (±SE) μmol P $mg^{-1}h^{-1}$DW at 0.11 mmol N l^{-1} and levelled off around this concentration. Results are shown in Appendix C (Table C.5) and Figure 4.16.

The experimental values for *C. vulgaris* could be fitted to the Michaelis-Menten equation with resulting V_{max} and K_m of 0.016 μmol P $mg^{-1}h^{-1}$DW and 0.009 mmolNl^{-1}, respectively. However, the calculated V_{max} was lower than the empirically measured rates of phosphate uptake. This could be explained by presence of inhibition effect on phosphate uptake by presence of high nitrate concentrations in the medium. The possible inhibition was observed at nitrogen concentrations above 2.28 mmol N l^{-1}.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.16 Rates of phosphate uptake by *S. subspicatus* (a) and *C. vulgaris* (b) grown with different initial concentrations of inorganic nitrogen Dashed lines represent (a) the average value rate of phosphate uptake, and (b) the Michaelis-Menten fitting to the experimental results Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.3.5 Discussion

In the present study nitrogen uptake was calculated as a decrease of nutrient concentration in the medium, and the assumption was made that the algal uptake is responsible for nutrient disappearance. It is well known that algae can accumulate nitrogen in excess of their requirements for steady-state growth rates: Richmond (1986) reported total nitrogen (TN) values ranging from 1 to 10% of algal dry weight. In cells of microalgae in the exponential growth phase, however, nitrogen accounts for about 7-10% of the dry weigh at an average carbon concentration of 50%. The uptake of nitrogen may therefore not be strictly coupled to growth, leading to poor relationships when modelling growth rate and substrate utilisation using Monod and Michaelis-Menten equations.

In these experiments nitrate was used as the sole nitrogen source. This is justified by the common ability of green algae, including *S. subspicatus* and *C. vulgaris*, to use NO_3^-, NO_2^- or NH_4^+ with similar values for maximum growth rates. For example, the values of maximum growth rate of *Chlorella ellipsoidea* and *Chlorella pyrenoidosa* grown on nitrate were 0.50 and 0.45 $\log_{10}$ units/day (Syrett 1981). In chemostat culture *Scenedesmus quadricauda* had a maximum N-specific growth rate of 1.4 d^{-1} (33.6 h^{-1}) grown on ammonium. Rhee (1978) reported that the maximum uptake rate

of nitrate in *Scenedesmus sp.* was 0.325×10^{-9} µM cell^{-1} min^{-1}. These values are similar to the half saturation constant used in the simulation for *Scenedesmus quadricauda* by Watanabe and Miyazaki (1996) of a linear relationship between specific growth rates. Other examples of N-specific growth rate are given in Appendix A.

Both species used in these experiments appeared to take up nitrogen independently of the initial carbon concentration and thus of the growth rate, indicating accumulation of tissue nitrogen under conditions of nitrate enrichment. High nitrogen uptake rates by both species may also be related to the high growth rates of Chlorophyceae (Sommer 1985; Happey-Wood 1989).

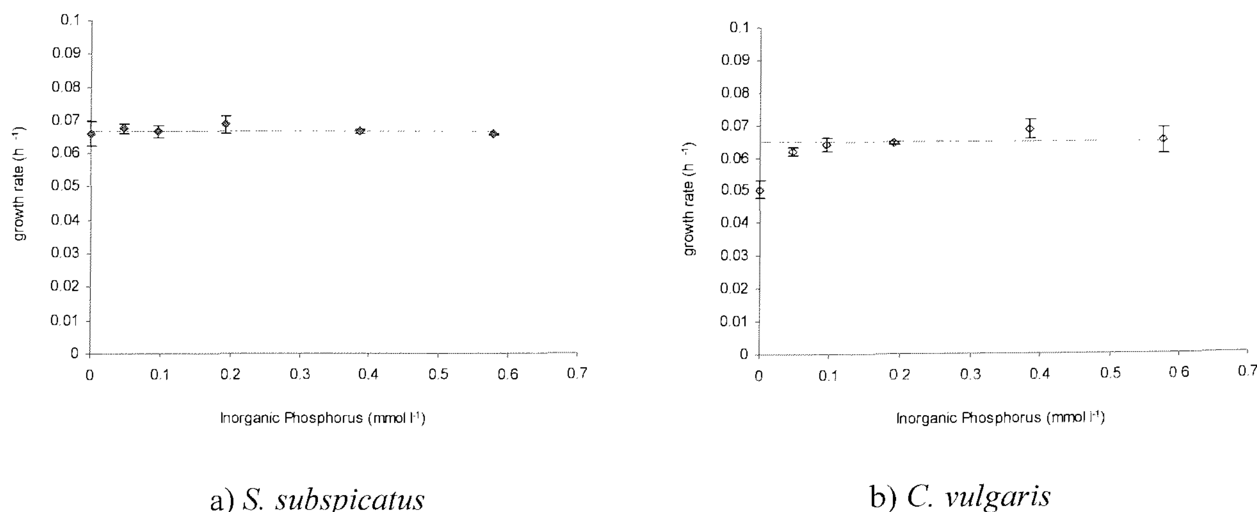
The Michaelis-Menten shape of nitrate uptake is probably a result of nitrate reductase (NR) activity. The enzyme is the first in the sequence of the nitrate assimilatory pathway and it is responsible for reduction of nitrate to nitrite for the biosynthesis of nitrogen-containing biomolecules. The highest NR specific activity found in the literature for *C. vulgaris* was reported by Solomonson *et al.* (1975). Among the factors responsible for nitrate assimilation into algal cells, the activity of NR is considered as the major factor, and in the turn the concentration of nitrate in the surrounding environment is the most important factor for regulation of NR activity. One of the common responses to exposure to high nitrate concentrations observed in fungi and bacteria is the induction of NR activity (Crawford & Arst 1993).

4.4 Effects of phosphorus enrichment on algal growth and activity

Growth characteristics, rates of nutrients uptake were assayed at phosphate concentrations ranging from 0 to 0.58 mmol l^{-1} using batch cultures of *S. subspicatus* and *C. vulgaris* (Table 4.14).

4.4.1 Growth

In the absence of detectable concentrations of PO_4^{2-} in the medium, cultures of *S. subspicatus* in the exponential phase grew on an average (n=3) at 0.066 h^{-1} and *C. vulgaris* at 0.050 h^{-1}. At higher concentrations, P-specific growth rates of *S. subspicatus* and *C. vulgaris* were approximately constant at 0.067 ± 0.000 (±SE) and 0.065 ± 0.001 (±SE) h^{-1}, respectively (Figure 4.17). Monod equation was applied to the results, but the fit was poor as there was still considerable growth at zero phosphorus concentrations.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.17 Effect of inorganic phosphorus concentration (mmol l^{-1}) on the specific growth rates of *S. subspicatus* (a) and *C. vulgaris* (b) cultures. Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

Table 4.14 Average values (n=3) of specific growth rates (μ) for *S. subspicatus* and *C.vulgaris* cultures grown with different concentrations of inorganic phosphorus in the medium.

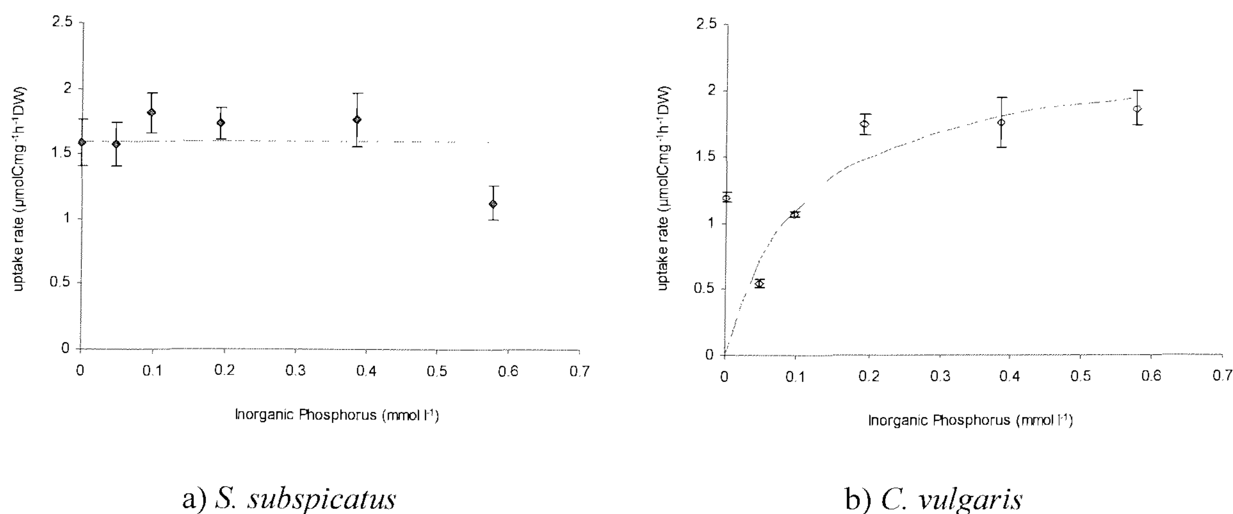
Phosphorus (mmol l^{-1})	μ (h^{-1})	
	S.subspicatus	*C.vulgaris*
0	0.066	0.050
0.05	0.067	0.062
0.10	0.067	0.064
0.19	0.069	0.064
0.39	0.067	0.068
0.58	0.066	0.065

4.4.2 Inorganic carbon uptake

The results for inorganic carbon uptake are given in Appendix C (Table C.6) and Figure 4.18. *S. subspicatus* showed no significant difference in carbon uptake for phosphate concentrations in the medium from zero to 0.58 mmol P l^{-1} with the average of 1.60 ± 0.10 (±SE) μmol P $mg^{-1}h^{-1}$DW. *C. vulgaris* showed the average carbon uptake of 1.79 ± 0.04 (±SE) at inorganic phosphorus levels above 0.1 mmol P l^{-1}. As might be expected, the Michaelis-Menten model gave rather a poor fit to the experimental results with *S.subspicatus*. When the phosphate limited growth rates and carbon uptake are compared it is clear that the growth and carbon uptake of *S. subspicatus* is unaffected by the lack of phosphate in the medium. The implication is that both species have some capacity for phosphate storage in their cells, and in the conditions used in these experiments this was sufficient for the growth and uptake needs of *S. subspicatus*.

There is however evidence of a small inhibition of growth rate and carbon uptake in *C. vulgaris* when phosphate is absent or at very low concentrations in the medium. The Michaelis-Menten model gave values of V_{max} and K_m equal to

2.32 μmolCmg^{-1}h^{-1} DW and 0.109 mmol P l^{-1}, respectively. Correlation coefficient between experimental and calculated values of inorganic carbon uptake was 0.72.

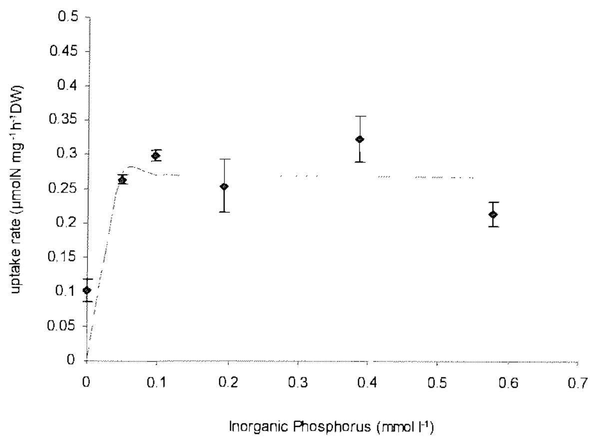


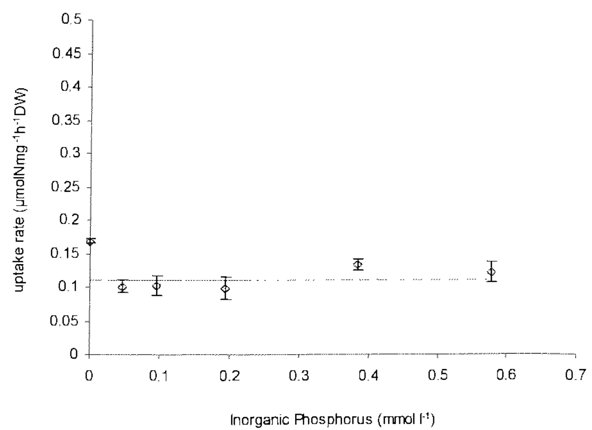
a) *S. subspicatus* b) *C. vulgaris*

Figure 4.18 Rates of inorganic carbon uptake by (a) *S. subspicatus* and (b) *C. vulgaris* cultures grown at different concentrations of inorganic phosphorus in the medium Dashed lines represent (a) the average value of phosphorus uptake rate, and (b) the Michaelis-Menten kinetic fitting to the experimental results Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.4.3 Nitrate uptake

With the exception of zero phosphorus in the medium, both species showed a fairly constant uptake of nitrate at all phosphate concentrations studied. The value of 0.270 ± 0.019 (±SE) μmol N mg^{-1}h^{-1}DW observed for nitrate uptake by *S. subspicatus* was more than double the rate of 0.111 ± 0.007 (±SE) μmol N mg^{-1}h^{-1}DW for *C. vulgaris* (Table C.7 and Figure 4.19). At zero phosphate concentrations there was evidence of a reduced nitrate uptake, which was more pronounced in *S. subspicatus* than in *C. vulgaris*. Experimental measurements again did not show a good agreement of experimental results for both species with the Michaelis-Menten model.





a) *S. subspicatus* b) *C. vulgaris*

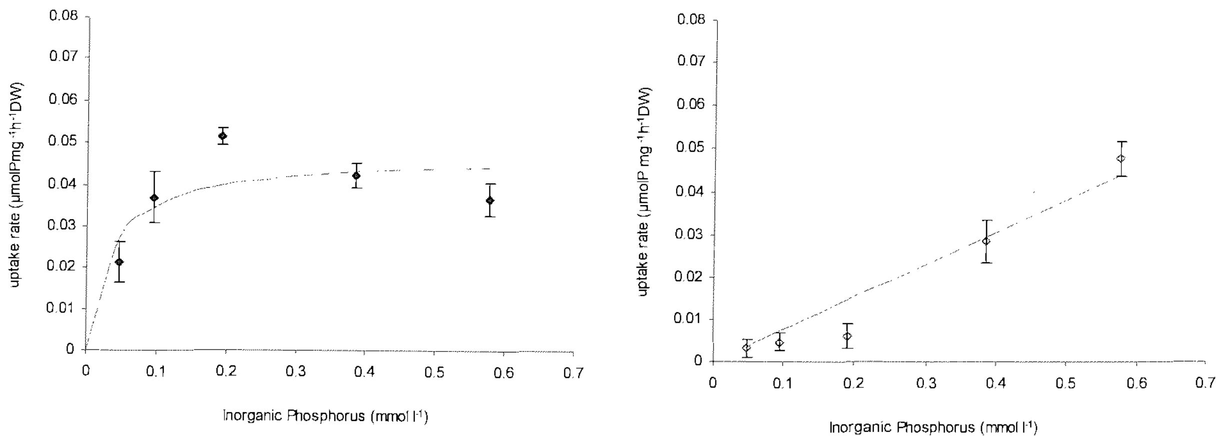
Figure 4.19 Rates of nitrate uptake by *S. subspicatus* (a) and *C. vulgaris* (b) cultures grown at different concentrations of inorganic phosphorus in the medium Dashed lines represent (a) the Michaelis-Menten kinetic fitting to the experimental results, and (b) the average value of phosphorus uptake rate Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.4.4 Inorganic Phosphorus uptake

Inorganic phosphorus uptake was measured by depletion of the nutrient in the medium. The results are shown in Figure 4.20 and Table C.8.

Experimental results for *S. subspicatus* showed a good fit to a Michaelis-Menten model (R^2=0.99), with a maximum phosphate uptake, V_{max} of 0.064 µmol Pmg^{-1} h^{-1} DW at an inorganic phosphorus concentration in the medium, K_m, of 0.34 mmol l^{-1}.

For *C. vulgaris*, however, the Michaelis-Menten model did not show a good fit due to almost linear increase in rates of phosphate uptake with increase of inorganic phosphorus concentrations in the growth medium. Thus the maximum rate of uptake for *C. vulgaris* was not observed.



a) *S. subspicatus* b) *C.vulgaris*

Figure 4.20 Rates of inorganic phosphorus uptake by *S. subspicatus* (a) and *C. vulgaris* (b) cultures grown at different initial concentrations of inorganic phosphorus. Dashed lines represent (a) the Michaelis-Menten kinetic fitting to the experimental results, and (b) the trend of phosphorus uptake rate Error bars indicate scattering in values among triplicates, not shown if smaller than symbol.

4.4.5 Discussion

With phosphorus as the limiting nutrient in the medium, growth rates remained approximately constant over the range of concentrations used, even when the available phosphate in the medium was zero. This suggests the cells have intracellular reserves that may be recycled over or long or short period. It is however clear that there is sufficient phosphate within the system to allow both species to grow exponentially during 32-40 hours with average doubling time of 10.3 and 10.7 hours for *S. subspicatus* and *C.vulgaris*, respectively. The relative growth rate for *S. subspicatus* was 0.067 h^{-1} and for *C. vulgaris* was 0.065 h^{-1}. The ability of the algae to grow at maximum rate at very low concentrations indicates the efficiency nutrient uptake and utilisation, reflected in the K_m values.

Algal nutrient uptake is a surface phenomenon (Smith & Kalff 1983) and any increase in cell surface: volume ratio will confer a competitive advantage to small phytoplankton. In the current work however *S. subspicatus* shows a significantly

higher uptake rate of both nitrate and phosphorus despite a biovolume (μm^3) of cell ratio of 5:1 between *S. subspicatus* and *C. vulgaris*, respectively (Bitton 1998).

In the present study in which the nutrient supply was as a single dose at the beginning of the growth phase, cultures of *S. subspicatus* showed higher phosphorus uptake rates than *C. vulgaris* cultures. Spijkerman & Coesel (1996) suggested that algae with higher maximum uptake rates of phosphorus have an advantage over algae with lower ones under phosphorus limitation. In a competitive situation between the two experimental species *S. subspicatus* would be expected to out-compete *C. vulgaris* when phosphorus is limited despite its slightly lower μ_{max} value.

There is considerable debate in the literature regarding the ecological significance of phosphorus uptake and factors that may affect it, including light, temperature, presence of other nutrients and pH (Kuenzler & Perras 1965; Kylin 1966; Fogg 1973). Results of these studies have not always been conclusive or in agreement. There is a general consensus, however, that phosphate is taken up to a greater extent than is required ('luxury consumption'). The availability of phosphate will however depend on the form in which it is present, and there is a preference for the orthophosphate form. A study of orthophosphate (^{32}P and ^{33}P) uptake by *Scenedesmus quadricauda* in batch cultures showed a general tendency of K_m and V_{max} values to increase as the concentration of external orthophosphate decreased. The results also indicated that at any stage of culture algae tend to release phosphorus compounds back into the medium (Lean & Nalewajko 1979) as cited by Berman (1964). As phosphate is a conservative molecule there is always a reservoir present in cells that can be recycled back into the growth medium. It is therefore very difficult in practice to have a culture that is truly phosphate-free even if the medium is formulated without phosphate, as the inoculum of cells could contain sufficient phosphate for the growth of several generations of new cells. According to John and Flynn (2000) polyphosphates occupying less that 1% of cell volume could support a generation of cell growth with little or no P-stress.

4.5 Experimental results for light-temperature effects on algal growth and activity

4.5.1 Light and temperature

Algal growth, rates of nutrients uptake and photosynthetic oxygen production were tested in batch cultures at the following combinations of irradiance and temperature: 78.3, 62.7, 47.0, 31.3, 15.7 and 7.8 μmol $m^{-2}s^{-1}$, and 5, 10, 15, and 20 °C, respectively.

Specific growth rates (μ) for *S. subspicatus* and *C. vulgaris* cultured at six light intensities and four temperatures are given in Table 4.15 and Table 4.16 as average values from four replicates, and plotted in Figure 4.21. Both *S. subspicatus* and *C. vulgaris* approached maximum growth rate at a light intensity of 47.0 μmol $m^{-2}s^{-1}$ at all temperatures tested. For both species the maximum growth rate increased with increasing temperature. From 5 °C to 15 °C the maximum growth rate for *C. vulgaris* was higher than for *S. subspicatus* at all light intensities. At 20 °C however values for *S. subspicatus* were higher than those for *C. vulgaris*. Growth rates for *C. vulgaris* showed relatively little change between 15 °C and 20 °C.

Table 4.15 Average values (n=4) of μ for *S. subspicatus* cultures grown at different light and temperature regimes.

Light (μmol $m^{-2}s^{-1}$)	μ (h^{-1})			
	at 20°C	at 15 °C	at 10 °C	at 5 °C
78.3	0.0626	0.0376	0.0222	0.0063
62.7	0.0647	0.0371	0.0193	0.0082
47.0	0.0644	0.0369	0.0163	0.0068
31.3	0.0461	0.0289	0.0136	0.0051
15.7	0.0250	0.0192	0.0131	0.0032
7.80	0.0138	0.0131	0.0076	0.0031

To provide a quantitative estimate of light limitation the experimentally obtained growth rates were fitted to the Monod equation. Calculated values for μ_{max}, I_K (is the half saturation constant i.e. the light intensity required to achieve half the maximum growth rate) and A are shown in Table 4.17.

The ratio μ_{max}/I_K is defined as the affinity (A), incorporates both parameters and is the initial slope of the uptake rate versus substrate concentration curve. A provides an index describing the ability of cells to accumulate substrate, while the parameter is independent of the uptake mechanism (Button 1985; Button 1986).

The initial slope of the curves decreases with decreasing temperature: one possible explanation might be a decrease in cellular chlorophyll content at lower temperatures (Kirk 1994). With decreasing temperatures light saturation occurs at lower light intensities, as indicated by Figure 4.21 and I_K values in Table 4.20, supporting the argument that μ_{max} is temperature-dependent.

Table 4.16 Average values (n=4) of μ, for *C. vulgaris* cultures grown at different light and temperature regimes.

Light (μmol $m^{-2}s^{-1}$)	μ (h^{-1}) at 20°C	at 15°C	at 10°C	at 5°C
78.3	0.0582	0.0482	0.0304	0.0121
62.7	0.0519	0.0516	0.0257	0.0120
47.0	0.0510	0.0500	0.0247	0.0101
31.3	0.0435	0.0451	0.0194	0.0096
15.7	0.0293	0.0321	0.0157	0.0061
7.80	0.082	0.0172	0.0039	0.0038

Figure 4.22 shows differences in response of μ_{max} values to temperature for *S. subspicatus* and *C. vulgaris*. Values of μ_{max} for *S. subspicatus* are exponential in form (R^2=0.99), with empirical expression:

$$\mu_{max} = 0.0046\ e^{0.1596\,T} \tag{4.2}$$

where T is expressed in °C. The best fit of μ_{max} for *C. vulgaris* is linear (R^2=0.98), however, with empirical expression:

$$\mu_{max} = 0.0042T \quad (4.3)$$

Table 4.17 Calculated values of μ, μ_{max}, and I_K for cultures of *S. subspicatus* and *C. vulgaris* grown at various temperatures.

Temperature (°C)	Species	μ_{max}	I_K	A
5	*S. subspicatus*	0.009	20.2	0.0004
	C. vulgaris	0.016	24.9	0.0006
10	*S. subspicatus*	0.026	20.2	0.0013
	C. vulgaris	0.042	35.0	0.0012
15	*S. subspicatus*	0.052	25.2	0.0021
	C. vulgaris	0.064	16.0	0.0040
20	*S. subspicatus*	0.103	39.5	0.0026
	C. vulgaris	0.081	33.8	0.0024

A statistical comparison (t-test) of algal growth rates for the two species grown in equal conditions showed that a significant difference exists at 20, 15 and 5 °C (P-value < 0.05), and only cultures at 10 °C showed a higher probability of similar growth response (Table 4.18).

In summary it appears that *C. vulgaris* is able to grow more vigorously than *S. subspicatus* at low temperatures and light intensities, but at temperatures above 15°C the growth rate of *S. subspicatus* continues to rise while that of *C. vulgaris* shows no further increase.

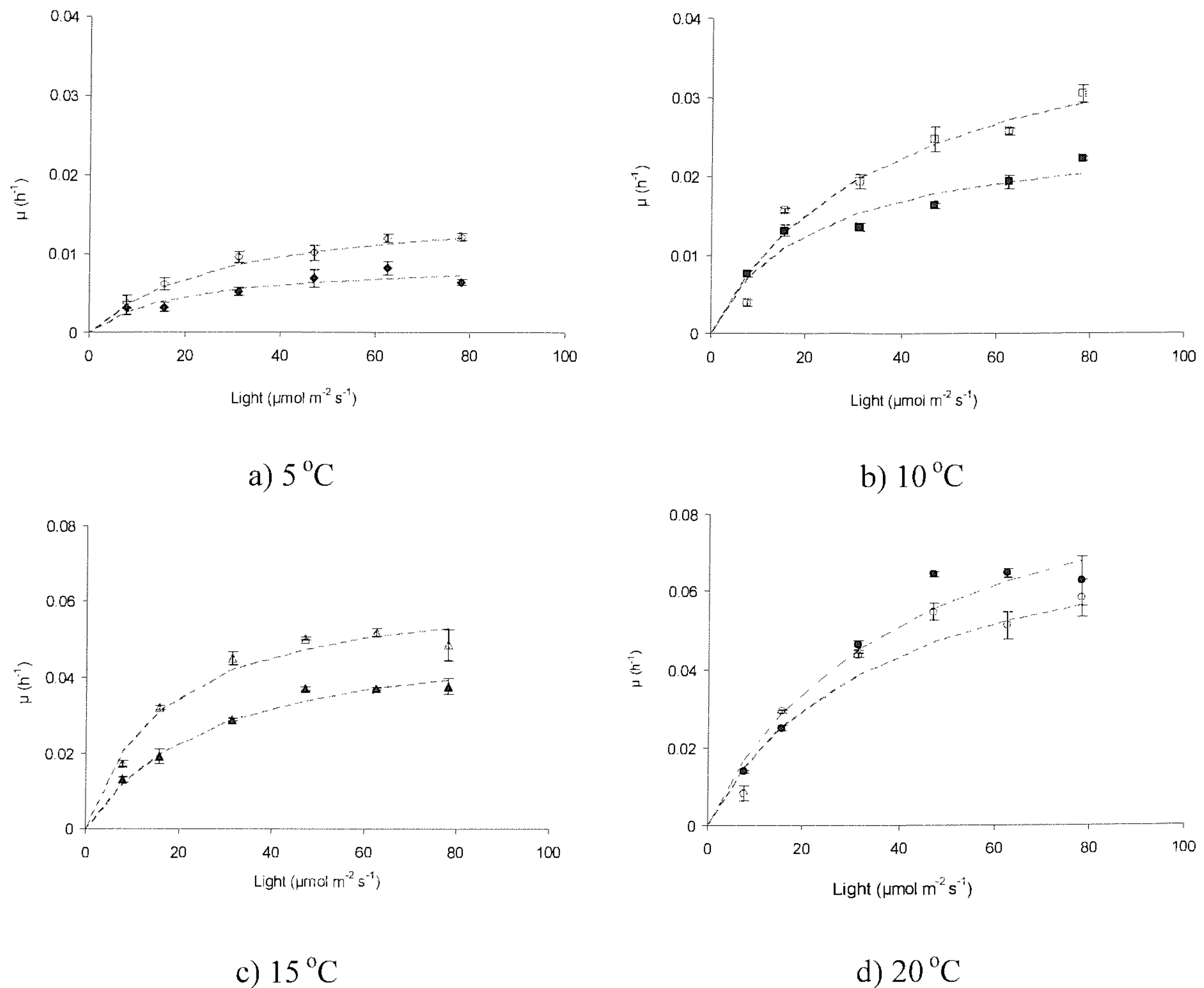
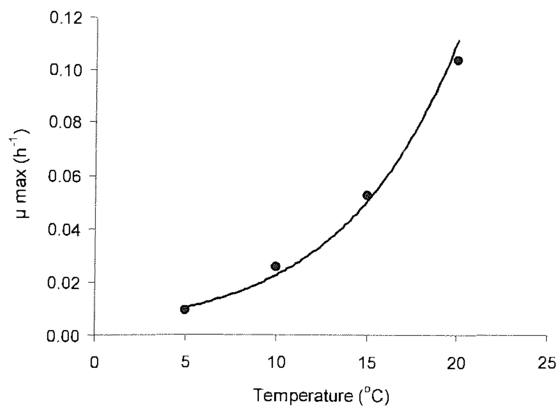
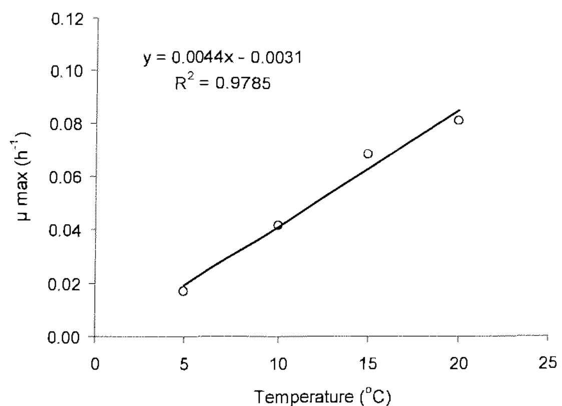


Figure 4.21 Effect of growth light intensity and temperature on the relative growth rate of *S.subspicatus* and *C. vulgaris* cultures. Dashed lines represent the Monod based fitting to empirical results. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

Figure 4.23 shows the growth rates of algae as a function of temperature for light intensities from 7.8 to 78.3 μmol $m^{-2}s^{-1}$. At all light intensities the two species of algae showed a similar response in growth to the tested range of temperatures (P-value higher than 0.05). The conclusions drawn from these graphs support the argument presented in the previous section: at light intensities up to 78.3 μmol $m^{-2}s^{-1}$ growth rates for *C. vulgaris* are higher than those for *S. subspicatus* at temperatures up to 15 °C.





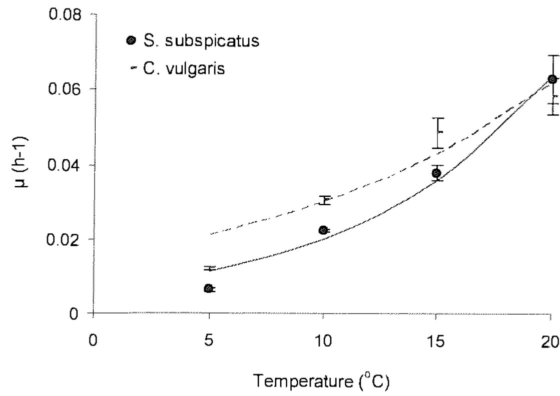
a) *S. subspicatus* b) *C. vulgaris*

Figure 4.22 Effect of temperature on calculated values of μ_{max} for (a) *S. subspicatus* and (b) *C. vulgaris* cultures.

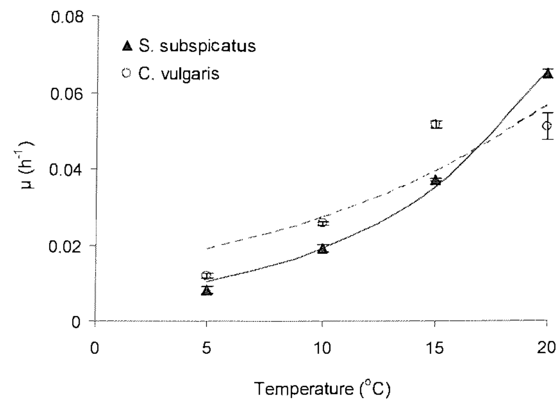
From Figure 4.23 it can be seen that growth rates for *S. subspicatus* increase with increasing light intensity at all temperatures up to a light intensity of 47.0 μmol $m^{-2}s^{-1}$. For *C. vulgaris* the effect of increasing light intensity on growth rate is smaller and there is no appreciable difference between light intensities greater than 31.3 μmol $m^{-2} s^{-1}$.

Table 4.18 Statistical comparison of growth rates for *S. subspicatus* and *C. vulgaris* cultures grown in equal conditions, using paired t-test.

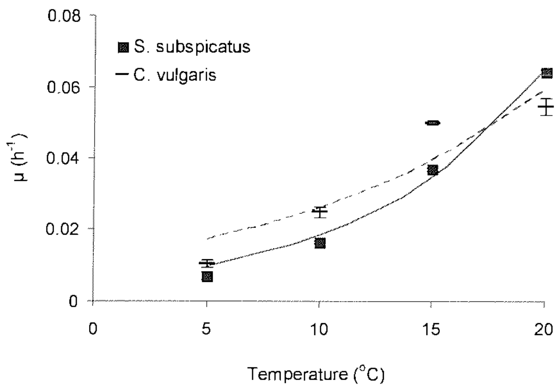
Temperature (°C)	20	15	10	5
P-value	0.037	0.001	0.056	0.004



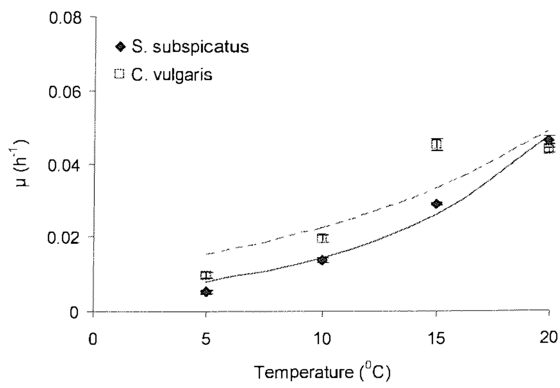
a) 7.8 μmol m^{-2} s^{-1}



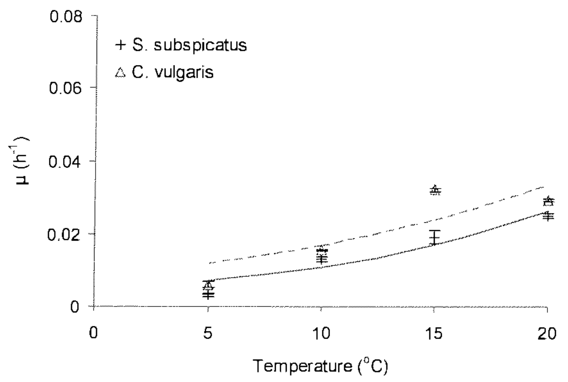
b) 15.7 μmol m^{-2} s^{-1}



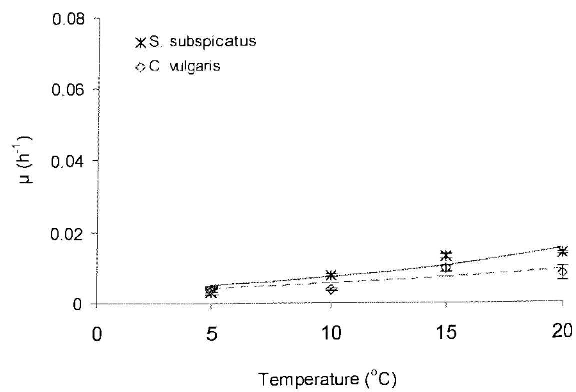
c) 31.3 μmol m^{-2} s^{-1}



d) 47.0 μmol m^{-2} s^{-1}



e) 62.7 μmol m^{-2} s^{-1}



f) 78.3 μmol m^{-2} s^{-1}

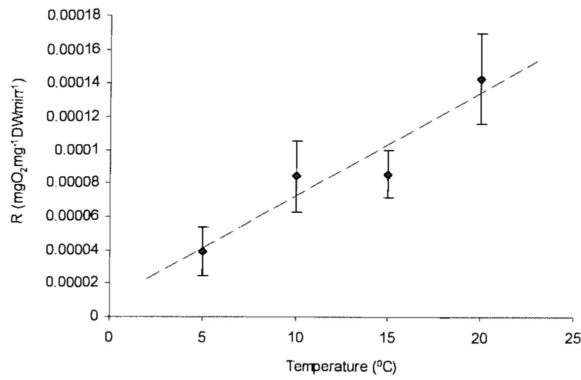
Figure 4.23 Growth rate of *S. subspicatus* and *C. vulgaris* as a function of temperature for different light intensities. The curves fits values were calculated using the equation (2.27). Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

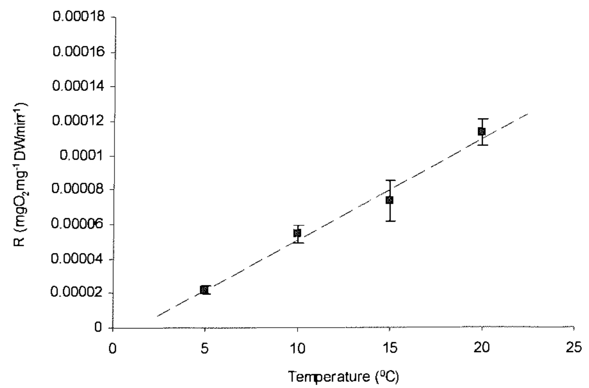
4.5.2 Respiration

There is considerable debate in the literature on whether dark respiration of algae should be used to calculate net photosynthesis from ^{14}C experiments. Thus, the experimental data on algal dark respiration is reported separately here.

In the first experiment cultures of *S. subspicatus* and *C. vulgaris* were grown at temperatures of 5, 10, 15, 20 °C at 78.32 μmol $m^{-2}s^{-1}$ of irradiance for 48 hours. The results showed there was an increase in respiration rates with increasing temperature, with the maximum in the range 0.00011-0.00014 mg O_2 $mg^{-1}min^{-1}DW$ for both species at 20 °C for the temperatures tested. Respiration rates of both *S. subspicatus* and *C. vulgaris* appeared to be linearly dependent on growth temperature (Figure 4.24), with R^2 values of 0.900 and 0.983 respectively, and a slope of approximately 6×10^{-6} mg O_2 $mg^{-1}min^{-1}DW$ per degrees °C. The temperature activity coefficient θ for respiration of both species was found to be 1.131.

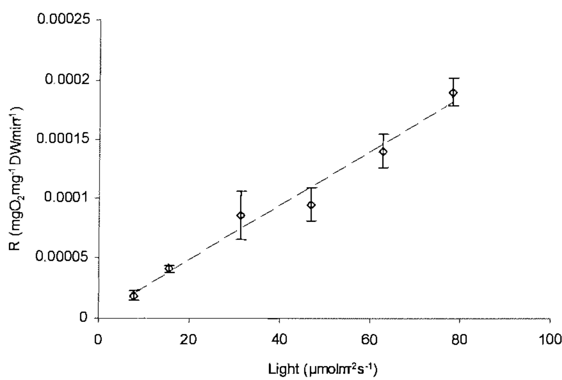
In the second experiment the two cultures were tested at six different light intensities of 78.32, 62.7, 47.0, 31.3, 15.7, 7.8 μmol $m^{-2}s^{-1}$ at 20 °C for 48 hours. The results showed an increase in respiration rates with light intensity, with the maximum in the range 0.00017-0.00019 mg O_2 $mg^{-1}min^{-1}DW$ for both species at 20 °C for the temperatures tested. Respiration rates of both *S. subspicatus* and *C. vulgaris* appeared to be linearly dependent on light intensity (Figure 4.25), with R^2 values of 0.976 and 0.988 respectively, and a slope of approximately 2×10^{-6} mg O_2 $mg^{-1}min^{-1}DW$ per μmol $m^{-2}s^{-1}$. Although much research assumes that the maximum dark respiration (photorespiration) to photosynthesis ratio is about 1:10, environmental factors do modify the actual ratio. The rate of algal respiration is not fixed: the previous light history is reported to have an influence over dark respiration of algal cells (Yallop 1982; Beardall *et al.* 1994). Temperature and respiration rates are directly related through the enzymatic activity of oxidative phosphorilation. The current work confirms that rates of respiration of exponentially growing cultures depend on the previous history of light exposure.

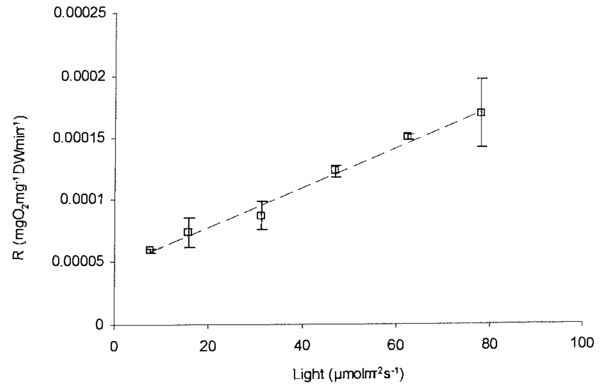




a) *S. subspicatus* b) *C. vulgaris*

Figure 4.24 Effect of growth temperature on respiration rates of *S. subspicatus* (a) and *C. vulgaris* (b). Error bars indicate scattering in values among replicates, not shown if smaller than symbol.





a) *S. subspicatus* b) *C. vulgaris*

Figure 4.25 Effect of light intensities on respiration rates of *S. subspicatus* (a) and *C. vulgaris* (b). Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

4.5.3 Photosynthesis

Rates of photosynthesis in *C. vulgaris* and *S. subspicatus* at all combinations of irradiance and temperature are shown in Figure 4.26. The results are in agreement with Verity (1981) who showed that the rate of photosynthesis varied markedly with growth temperature under light limiting as well as under light-saturated conditions.

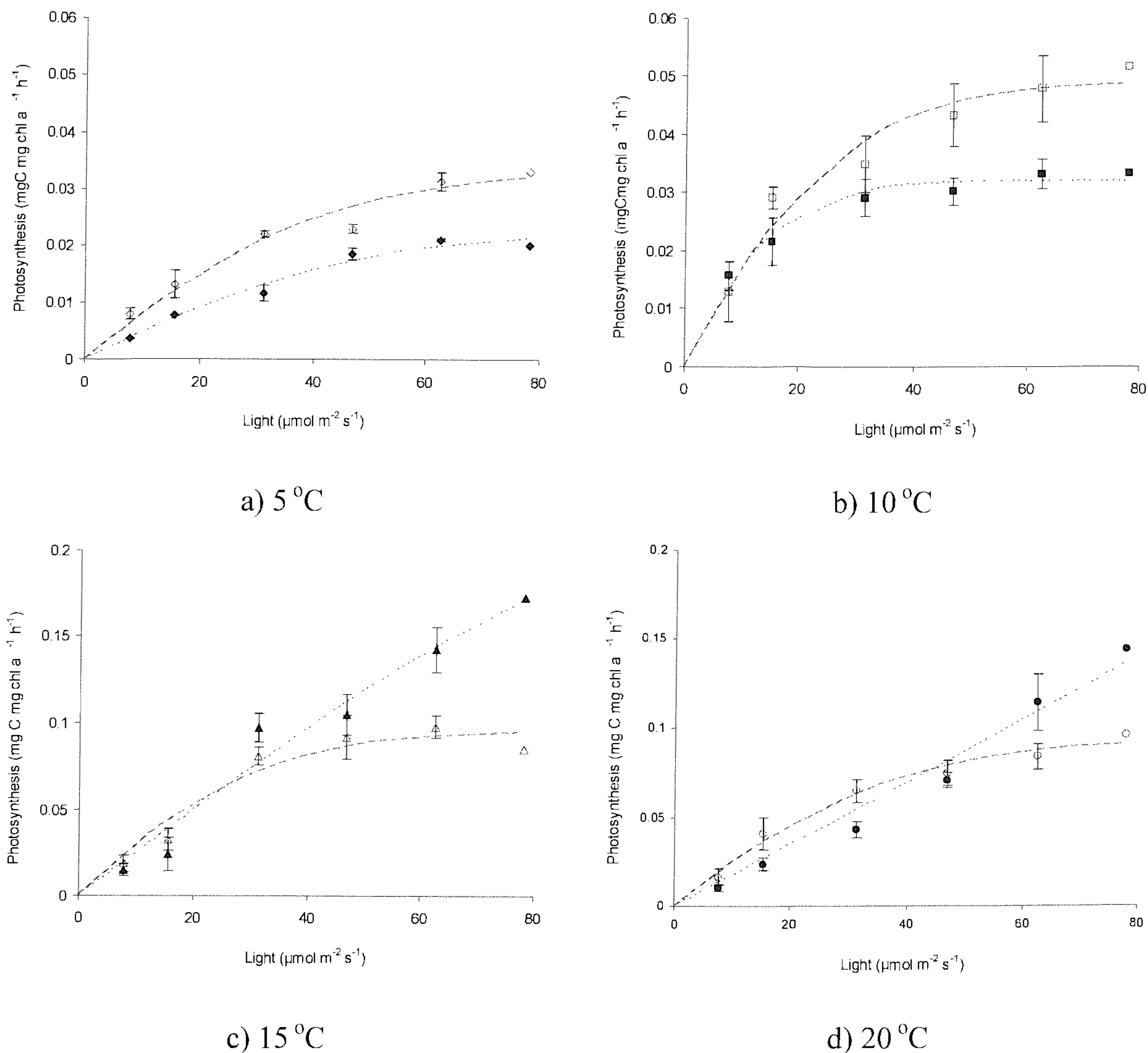


Figure 4.26 Rates of photosynthesis by *S. subspicatus* (closed symbols) and *C. vulgaris* (open symbols) at different light intensities. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

At 5 °C photosynthesis rates of *C. vulgaris* cultures were clearly higher than *S. subspicatus* at all levels of irradiance (Figure 4.26a). Both species showed an approximation to saturation at higher light intensities, with average values of 0.020 and 0.032 mg C mg^{-1} h^{-1} DW for *S. subspicatus* and *C. vulgaris*, respectively.

At 10 °C *C. vulgaris* species were again dominant in carbon fixing (Figure 4.26b) although saturation light intensities appeared to be lower than at 5 °C. The beginning of the saturation plateau was 31.3 µmol m^{-2} s^{-1} with an average photosynthetic rate of 0.031 mg C $mg^{-1}h^{-1}$chl **a** for *S. subspicatus* and 47.0µmol $m^{-2}s^{-1}$ with an average photosynthetic rate of 0.042 mg C $mg^{-1}h^{-1}$chl **a** for *C. vulgaris*.

At 15 °C both species showed similar photosynthetic rates at lower light intensities, namely between 7.8 and 47.0 μmol $m^{-2}s^{-1}$ (Figure 4.26c). While *C. vulgaris* exhibited saturation at 47.0 μmol $m^{-2}s^{-1}$ with an average value of 0.091 mg C $mg^{-1}h^{-1}$chl**a**, however, the photosynthetic rates of *S. subspicatus* cultures appeared to continue increasing above the value of 0.173 mg C $mg^{-1}h^{-1}$chl **a** at the highest irradiance tested. The saturation point for *S. subspicatus* must therefore lie above 78.3 μmol $m^{-2}s^{-1}$.

At 20 °C *S. subspicatus cultures* showed a steady increase in rates of photosynthetic carbon fixation at all light intensities applied (Figure 4.26d). *C.vulgaris* cultures, on the other hand, reached a plateau from 47.0 μmol $m^{-2}s^{-1}$, with an average rate of around 0.085 mg C $mg^{-1}h^{-1}$chl **a** at the highest light intensities used.

When the results for each species are compared (Figure 4.26), it can be seen that the highest rates for both species were recorded at 15 °C, indicating that this was the optimum temperature for photosynthesis. *S. subspicatus* rates of photosynthetic carbon fixation at 5 and 10 °C had clear light intensity saturation values of 47.0 and 31.3 μmol $m^{-2}s^{-1}$, respectively. At higher temperatures of 15 and 20 °C, however, the species did not show saturation at the light intensities applied, leading to the conclusion that the optimum levels of irradiance must be higher than 78.3 μmol $m^{-2}s^{-1}$. *C. vulgaris* cultures reached saturation at all temperatures tested, and there was little difference between photosynthetic rates at 15 °C and 20 °C. These results add support to the previous conclusions that *C. vulgaris* is better adapted to growth at low temperature and light intensities, but may be out-competed by *S.subspicatus* in warmer brighter conditions.

4.5.3.1 Photosynthesis-irradiance curve fitting

Experimentally obtained data showed no indications of photoinhibition. The equation proposed by Jassby and Platt (1976) was used to fit parameter values for *PI* curves (Table 4.19). Statistical comparison of experimental and calculated datasets showed no significant differences indicating a good fit to the empirical curves relating photosynthetic rate and irradiance.

The highest rates of photosynthesis were calculated for *S. subspicatus* cultures grown at 15 and 20 °C. Between the two species, higher values of photosynthetic efficiency, α, were generally found in cultures of *C. vulgaris*, indicating greater efficiency at utilising light at low intensities.

Table 4.19 Calculated parameters for *PI* curves (P_{max} and α) for *S. subspicatus* and *C. vulgaris*.

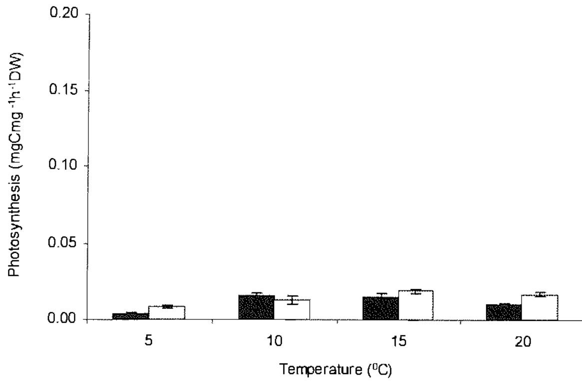
Temp. (°C)	*S. subspicatus*		*C. vulgaris*	
	P_{max}	α	P_{max}	α
5	0.023	0.0005	0.034	0.0008
10	0.032	0.0018	0.049	0.0017
15	0.286	0.0025	0.097	0.0030
20	-	-	0.094	0.0025

Table 4.20 P-values for experimental and calculated *PI* curves for *S. subspicatus* and *C. vulgaris* cultures.

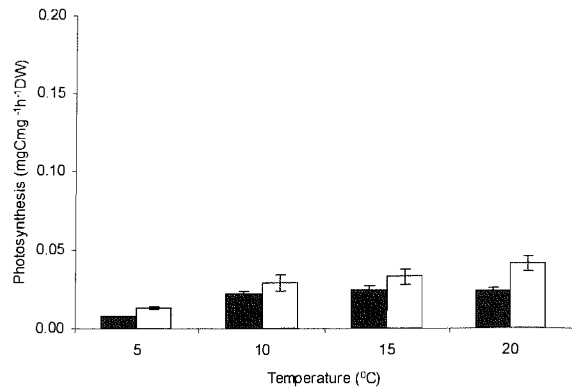
P-values	Temperature (°C)			
	5	10	15	20
S. subspicatus	0.997	0.922	0.986	0.930
C. vulgaris	0.994	0.962	0.954	0.997

4.5.4 Temperature

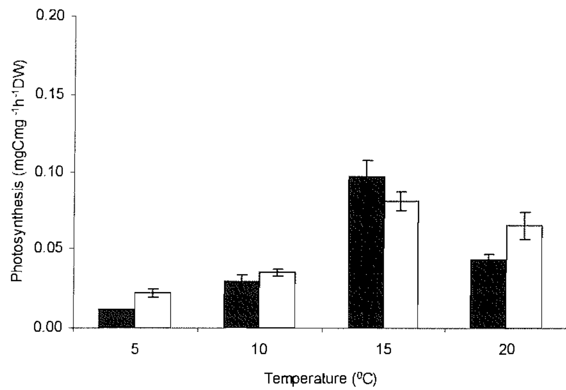
Rates of photosynthetic carbon uptake revealed a significant response to temperature in both species (Figure 4.27). Once again the optimum temperature for both species at all light intensities appears to be 15 °C, although the difference is more pronounced at higher light intensities. Some photosynthesis occurs even at very low light levels but temperature effects are small in this case, indicating that light is the limiting factor.



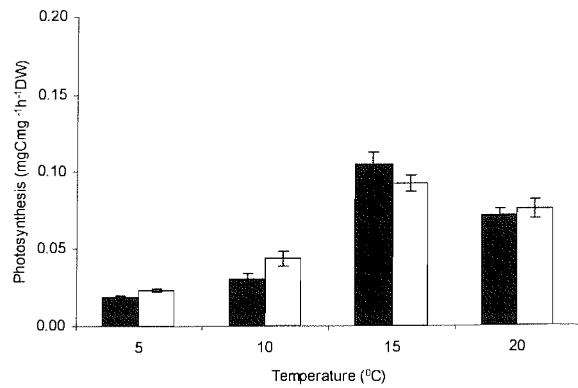
a) 7.8 μmol m^{-2} s^{-1}



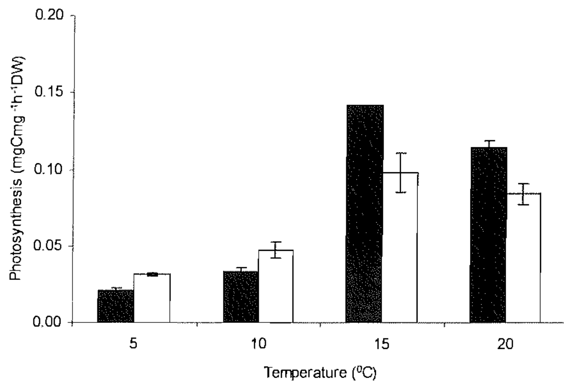
b) 15.7 μmol m^{-2} s^{-1}



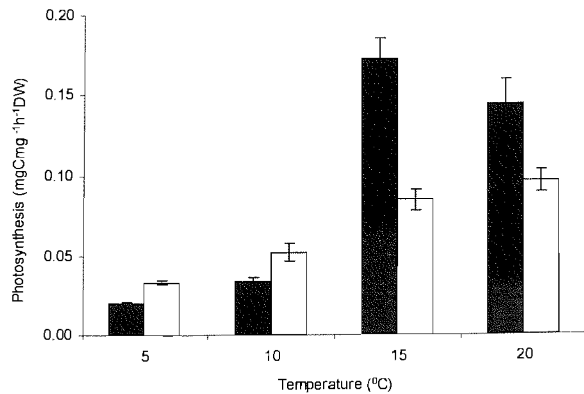
c) 31.3 μmol m^{-2} s^{-1}



d) 47.0 μmol m^{-2} s^{-1}



e) 62.7 μmol m^{-2} s^{-1}



f) 78.3 μmol m^{-2} s^{-1}

Figure 4.27 Photosynthetic rates of *S. subspicatus* (black) and *C. vulgaris* (white) at four temperatures and six light intensities. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

ANOVA analysis of variance showed that the photosynthetic rate of *S. subspicatus* cultures grown at four different temperatures was significantly different ($F_{1,5}$= 4.65). For *C. vulgaris* cultures grown under the same conditions, ANOVA also revealed a significant difference ($F_{1,5}$= 5.05).

4.5.5 Nitrogen uptake

Specific rates of NO_3 uptake by cultures of *S. subspicatus* and *C. vulgaris* grown in different light and temperature conditions are shown in Table 4.21 and Table 4.22.

Table 4.21 Specific rate of NO_3 uptake by *S. subspicatus* cultures grown in different light and temperature conditions.

Light	V_{max} (μmol N $mg^{-1}h^{-1}$DW)			
(μmol $m^{-2}s^{-1}$)	at 20 °C	at 15 °C	at 10 °C	at 5 °C
7.80	0.090	0.086	0.089	0.074
15.7	0.138	0.154	0.103	0.069
31.3	0.152	0.148	0.152	0.086
47.0	0.188	0.0158	0.158	0.089
62.7	0.160	0.144	0.183	0.079
78.3	0.217	0.124	0.189	0.090
V_{max}	0.222	0.156	0.219	0.088
K_I	11.2	3.6	14.5	2.1
A (V_{max}/ K_I)	0.020	0.043	0.015	0.043

4.5.5.2 Effect of light on rates of algal nitrate uptake

As shown in Figure 4.28 rates of nitrate uptake by *S. subspicatus* and *C. vulgaris* cultures during the exponential growth phase demonstrated saturation kinetics at all four temperatures. Neither species however showed considerable increase in nitrate uptake with light intensity at 5 °C. Both species showed a similar response to the applied experimental conditions. *S. subspicatus* has higher rates of uptake compared to *C. vulgaris* at 10 °C, 15 °C, and 20 °C. At 20 °C nitrate uptake increases just under 3 times with a ten-fold increase in light intensity for both species. The relationship between nitrate uptake and light intensities fits the Michaelis-Menten equation, suggesting that light has a limiting effect on nitrate uptake by the algae. The uptake of

nitrate is an energy-dependent process (Brown & Johnson 1977; Wood & Flynn 1995), and results of this type are therefore to be expected since temperature and light affect both the energy production and utilisation efficiency of the cell.

As expected the V_{max} values for *S. subspicatus* rose gradually but steadily between 0.074 and 0.090 μmol N $mg^{-1}h^{-1}DW$ in response to increased temperature. The same was true for C. *vulgaris* where V_{max} values rose from 0.070 to 0.083 μmol N $mg^{-1}h^{-1}DW$. Calculated values of A, as an ability of cells to accumulate substrate, show that 10°C was the least favourable temperature for both species in nitrate uptake.

Table 4.22 Specific rate of NO_3 uptake by *C. vulgaris* cultures grown in different light and temperature conditions.

Light	V_{max} (μmol N $mg^{-1}h^{-1}DW$)			
(μmol $m^{-2}s^{-1}$)	at 20 °C	at 15 °C	at 10 °C	at 5 °C
7.80	0.083	0.069	0.061	0.070
15.7	0.124	0.097	0.069	0.089
31.3	0.130	0.107	0.093	0.105
47.0	0.137	0.116	0.102	0.106
62.7	0.187	0.120	0.136	0.093
78.3	0.204	0.118	0.129	0.109
V_{max}	0.209	0.122	0.157	0.112
K_I	13.03	4.19	17.83	4.28
A (V_{max}/ K_I)	0.016	0.029	0.009	0.026

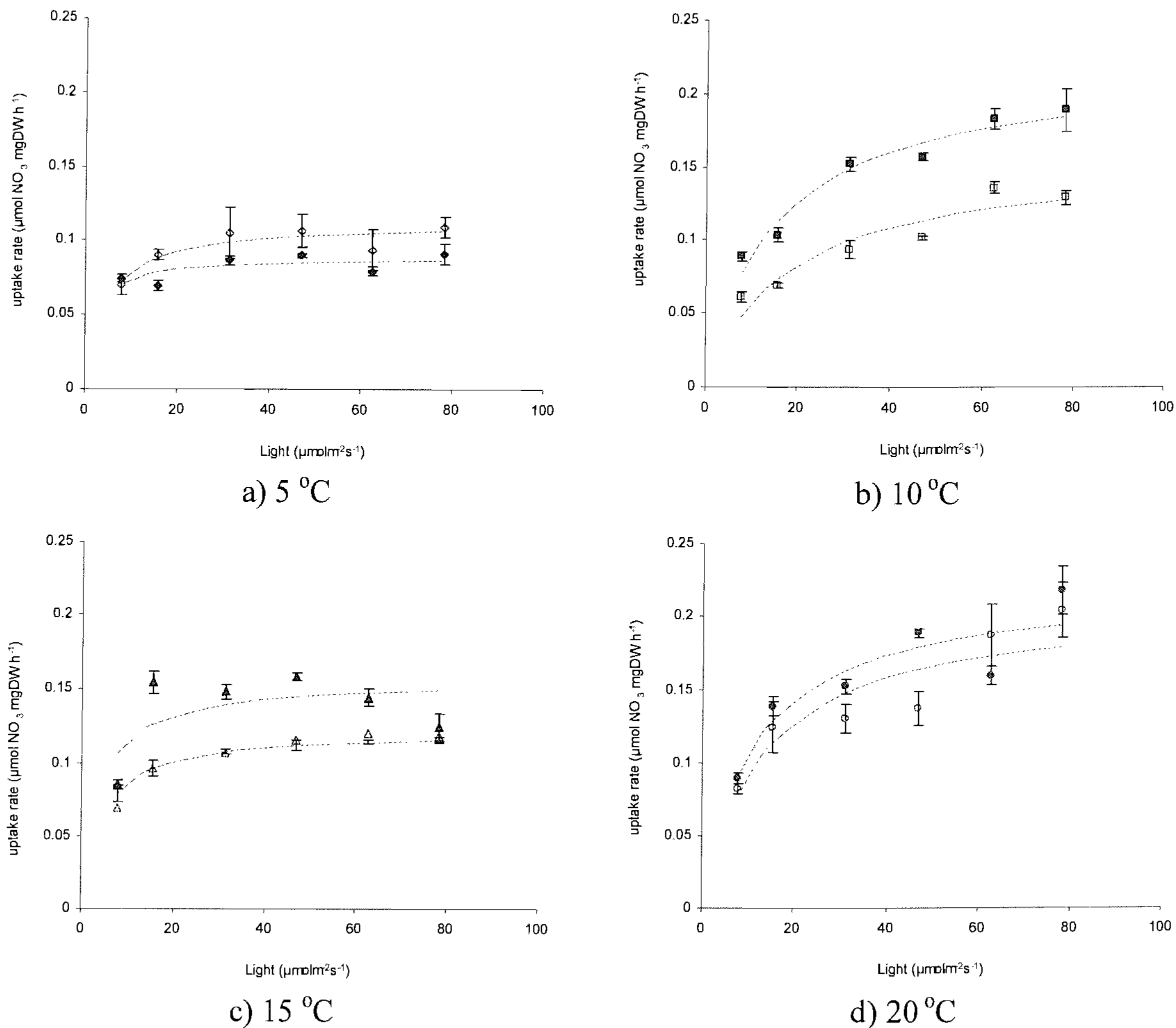


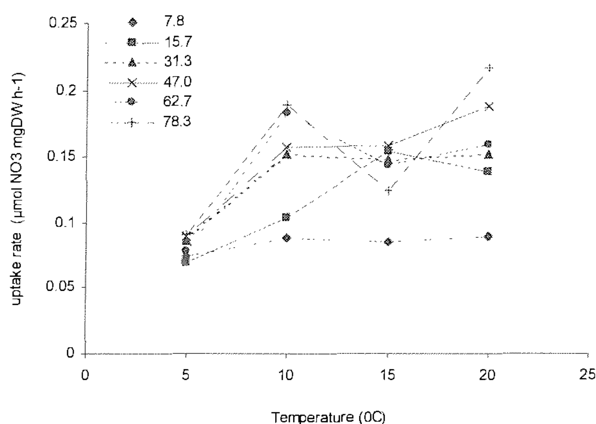
Figure 4.28 Rates of NO_3 uptake by *S. subspicatus* (closed symbols) and *C. vulgaris* (open symbols) during exponential growth at different temperatures as a function of light intensity. Data presented as average (n=4); dashed lines are calculated rates. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

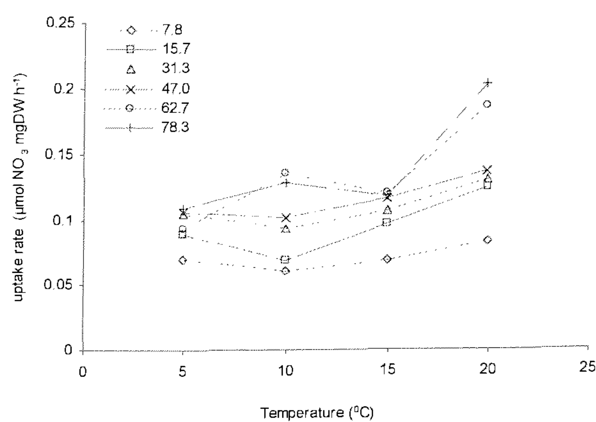
4.5.5.3 Temperature effects on rates of nitrate uptake by algae

Both species show little change in nitrate uptake at low light intensities in the temperature range studied. Table 4.23 shows the results of calculations of nitrate kinetics for *S.subspicatus* and *C.vulgaris* using the equation 2.27. With increasing light intensity there is a corresponding increase in nitrate uptake indicating the temperature dependence of the process. In the range considered the response of nitrate uptake to temperature appears to be approximately linear with θ of 1.03.

Table 4.23 Nitrate uptake kinetics for *S. subspicatus* and *C. vulgaris* in relation to temperature.

Light (μmol $m^{-2}s^{-1}$)	*S.subspicatus*			*C.vulgaris*		
	r_{20}	θ	correlation coefficient	r_{20}	θ	correlation coefficient
7.8	0.078	1.015	0.70	0.091	1.010	0.78
15.7	0.118	1.032	0.78	0.154	1.042	0.84
31.3	0.124	1.018	0.78	0.162	1.027	0.74
47.0	1.33	1.020	0.90	0.193	1.039	0.89
62.7	0.177	1.042	0.88	0.170	1.026	0.56
78.3	0.188	1.044	0.85	0.205	1.042	0.70





a) *S. subspicatus* b) *C. vulgaris*

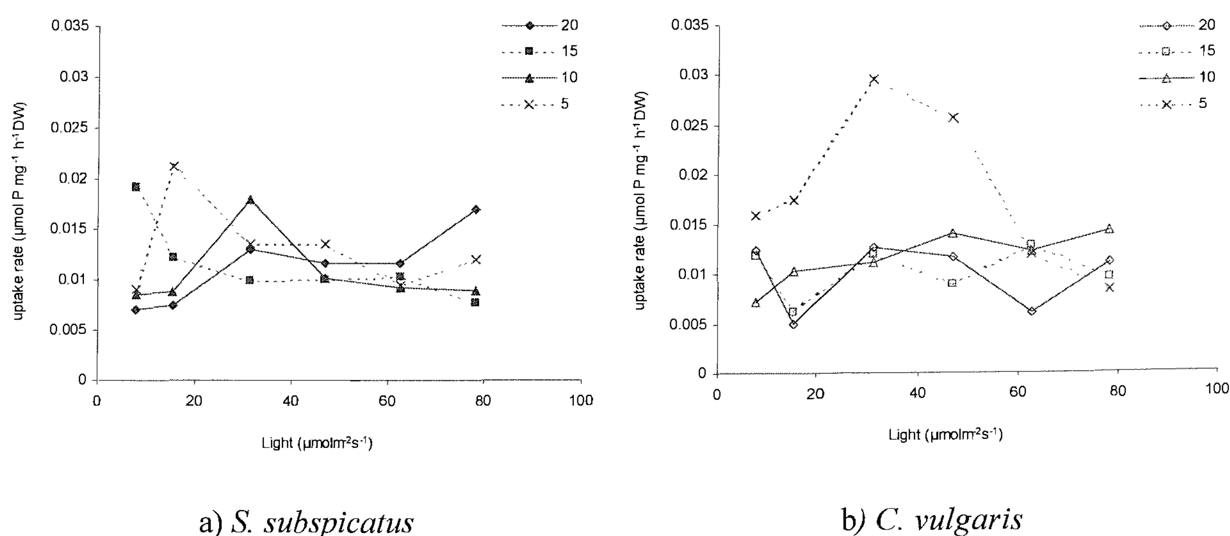
Figure 4.29 Temperature effect on NO_3^- uptake *S. subspicatus* and *C. vulgaris* cultures grown at different light intensities. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

4.5.6 Phosphorus uptake

Specific rates of PO_4 uptake by cultures of *S. subspicatus* and *C. vulgaris* grown in different light and temperature conditions are shown in Table 4.24 and Table 4.25.

4.5.6.1 Effect of light and temperature on rates of algal phosphorus uptake

At 5 °C, cultures of C. vulgaris appeared to show slightly decreasing P-uptake rates with increase in growth light intensity, from 0.016 to 0.008 μmol P $mg^{-1}h^{-1}$DW. *S.subspicatus* cultures however did not exhibit any trend in phosphate uptake rate; the average value for P-uptake was 0.013 μmol P $mg^{-1}h^{-1}$DW. Stable rates of P-uptake were also observed in cultures grown at 10 °C (Figure 4.30), with an average of 0.011 μmol P $mg^{-1}h^{-1}$DW for *S. subspicatus*, and of 0.012 μmol P $mg^{-1}h^{-1}$DW for *C.vulgaris*. At 15 °C, P-uptake for *S. subspicatus* showed no decrease in values apart from the cultures which grew at 7.8 μmol $m^{-2}s^{-1}$ where the average rate between four replicates reached 0.019 μmol P $mg^{-1}h^{-1}$DW . Rates of P-uptake for *C. vulgaris* at 15°C, on the other hand, appeared to be independent of light intensity. Cultures of *S.subspicatus* grown at 20 °C appeared to show a generally increasing rate of uptake with rising in light intensity.



a) *S. subspicatus* b) *C. vulgaris*

Figure 4.30 Rates of PO_4 uptake by *S. subspicatus* (closed symbols) and *C. vulgaris* (open symbols) during exponential growth as a function of light intensity. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

Table 4.24 Rates of phosphate uptake for *S. subspicatus* cultures grown under different light and temperature conditions.

Light (μmol $m^{-2} s^{-1}$)	V_{max}			
	at 20°C	at 15°C	at 10°C	at 5°C
7.8	0.007	0.019	0.009	0.009
15.7	0.007	0.012	0.009	0.021
31.3	0.013	0.010	0.018	0.014
47.0	0.012	0.010	0.010	0.014
62.7	0.012	0.010	0.009	0.009
78.3	0.017	0.008	0.009	0.012

Analysis of variance showed that the rates of P-uptake for *S. subspicatus* and *C. vulgaris* were not significantly different in response to different temperatures (Table 4.26) or different light intensities (Table 4.27).

Table 4.25 Rates of phosphate uptake for *C. vulgaris* cultures grown at different light and temperatures.

Light (μmol $m^{-2}s^{-1}$)	V_{max}			
	at 20°C	at 15°C	at 10°C	at 5°C
7.8	0.011	0.012	0.007	0.016
15.7	0.005	0.006	0.010	0.018
31.3	0.013	0.012	0.011	0.029
47.0	0.012	0.009	0.014	0.026
62.7	0.006	0.013	0.012	0.012
78.3	0.011	0.010	0.014	0.008

Table 4.26 Analysis of variance (ANOVA) of P-uptake rates between *S. subspicatus* and *C. vulgaris* cultures grown at different temperatures.

Temperature (°C)	5	10	15	20
P-value	0.21	0.62	0.51	0.48

Table 4.27 Analysis of variance (ANOVA) of P-uptake rates between *S. subspicatus* and *C. vulgaris* cultures grown at different light intensities.

Light (μmol $m^{-2}s^{-1}$)	7.8	15.7	31.3	47.0	62.7	78.3
P-value	0.594	0.303	0.669	0.663	0.820	0.614

4.5.7 Discussion

For both species the maximum growth rate increased with increasing temperature. Both *S. subspicatus* and *C. vulgaris* approached maximum growth rate at a light intensity of 47.0 μmol $m^{-2}s^{-1}$ at all temperatures tested. Calculations of μmax resulted in empirical expressions 4.1 and 4.2 for *S.subspicatus* and *C.vulgaris*, respectively.

C. vulgaris grew more vigorously than *S. subspicatus* at low temperatures and light intensities, but at temperatures above 15°C the growth rate of *S. subspicatus* continues to rise while that of *C. vulgaris* shows no further increase. In practical terms this means that in the natural environment *C. vulgaris* may appear earlier and compete more effectively in early spring but may lose its advantage as the temperatures and light intensity increases in to the summer period.

Respiration rates of both *S. subspicatus* and *C. vulgaris* depended linearly on growth temperature, with θ =1.131. Respiration rates of both *S. subspicatus* and *C. vulgaris* appeared to be linearly dependent on light intensity with a slope of around 2×10^{-6} mg O_2 $mg^{-1}min^{-1}$DW per μmol $m^{-2}s^{-1}$of light intensity. The rate of algal respiration is not fixed: the previous light history is reported to have an influence over dark respiration of algal cells (Yallop 1982; Beardall *et al.* 1994). Temperature and respiration rates are directly related through the enzymatic activity of oxidative phosphorilation. The

current work confirms that rates of respiration of exponentially growing cultures depend on the previous history of light exposure.

The highest rates of photosynthesis were recorded at 15 °C for both species, indicating that this was the optimum temperature for photosynthesis. Between the two species, higher values of photosynthetic efficiency, α, were generally found in cultures of *C. vulgaris*, indicating greater efficiency at utilising light at low intensities. Results support to the previous conclusions that *C. vulgaris* is better adapted to growth at low temperature and light intensities, but may be out-competed by *S.subspicatus* in warmer brighter conditions.

The relationship between uptake rates of nitrogen and light for *S. subspicatus* and *C. vulgaris* fits the Michaelis-Menten equation, suggesting that light had a limiting effect on nitrate uptake in batch cultures. Both species showed the tendency for V_{max} and K_I to increase as the light intensity was increased from 7.8 to 78.3 μmol $m^{-2}s^{-1}$. Nitrate uptake is an energy-dependent process (Brown & Johnson 1977; Wood & Flynn 1995), and results of this type are therefore to be expected since temperature and light affect both the energy production and utilisation efficiency of the cell.

Algal phosphorus uptake is the active process; the energy is supplied either from photosynthesis by photosynthetic phosphorilation or respiration by oxidative phosphorilation. Rates of phosphorus uptake were found to be light dependent in blue green alga *Anabaena cy*lindrica (Talpasayi 1962) and in green alga *Ankistrodesmus braunii* (Simonis & Urbach 1963) at substantial concentrations of phosphate in medium. The same authors reported a stimulation effect of temperature on phosphate uptake. However, Kylin (1966) reported results of studies with Scenedesmus sp. grown in the medium with high concentrations of CO_2, where the stimulation effect of temperature was not pronounced clearly. As phosphate uptake depends on photosynthetic phosphorylation and is in competition with I the photosynthetic carbon fixation cycle for energy supply. The current results support this finding for both algal species.

It is interesting that the optimum temperature for growth and photosynthesis of *S.subspicatus* and *C.vulgaris* at light intensities higher than 30 μmol$m^{-2}s^{-1}$ was 15 °C and is different from nitrate uptake (20°C). For phosphate uptake the optimum

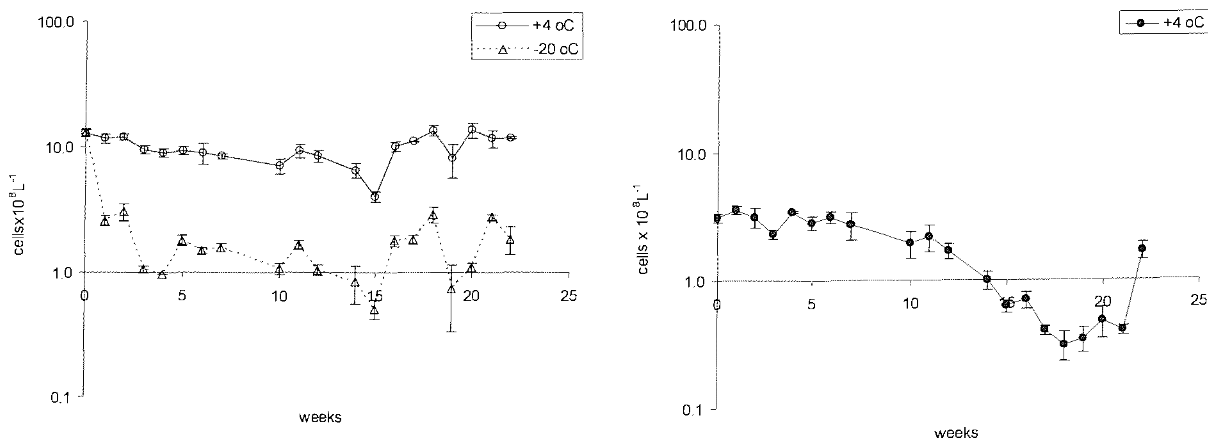
temperature was not identified as the rates of uptake were varied at approximately (with the exclusion of *C.vulgaris* cultures grown at 5 °C). Such differences were also reported for *Chlorella pyreidosa* (Shelef *et al.* 1970) and *Codium fragile* (Hanisak & Harlin 1978). These differences may be explained by the fact that optimum temperatures for enzyme reactions are not the same as those for growth (Innis & Ingraham 1978). The uncoupling between growth and nutrient uptake may be of ecological significance, because the two different temperature optima would effectively widen the temperature range for survival.

4.6 Results on survival of algae in complete darkness at low temperatures

Cultures of *S. subspicatus* and *C. vulgaris* were maintained stationary in complete darkness at +4 °C (group 1) and -20 °C (group 2) for up to 22 weeks, in replicate containers resuscitated and analysed at weekly intervals.

4.6.1 Plating

The number of colonies developed from a container is assumed to represent the amount of cells remaining viable and is given in Figure 4.31. Cells of *C. vulgaris* cultures stored at +4 °C in complete darkness showed no significant loss of viability, with an average survival rate of 74% over the experimental period. *C.vulgaris* cells stored at -20 °C in complete darkness showed a rapid initial loss of viability, which stabilised at a survival rate of around 12% for the remainder of the experimental period (Figure 4.31a). *S.subspicatus* cultures stored at +4 °C in complete darkness showed an average survival rate of 99% during the first 8 weeks of the experiment followed by a decrease during the following weeks which stabilised at about 12% in the final 4-5 weeks of the experiment (Figure 4.31b). For *S. subspicatus* cultures stored at -20 °C in complete darkness (group 2) zero recovery was found during the entire experiment.



a) *C. vulgaris* group1 and 2 b) *S. subspicatus* group 1

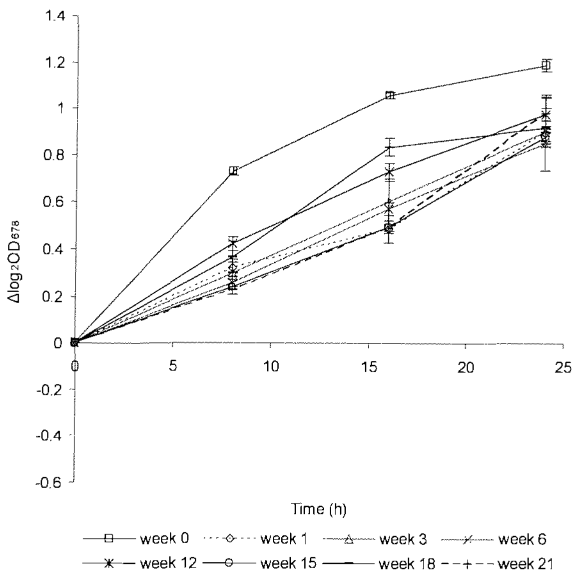
Figure 4.31 Viability of *C. vulgaris* group 1 and 2 (a) and *S. subspicatus* group 1 (b) cultures expressed as number of cells $\times 10^8$ l^{-1}. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

4.6.2 Growth response curves

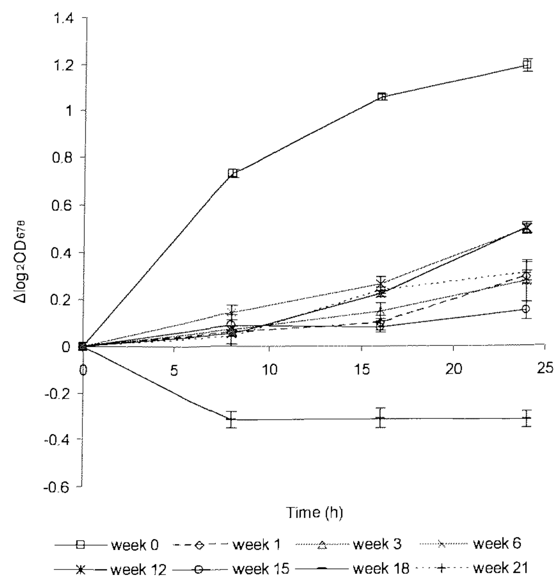
Growth response curves for group 1 and group 2 of *C. vulgaris* and *S. subspicatus* experimental cultures were calculated each week over the experimental period. Figure 4.32 shows some examples of the change in growth response curves for all groups.

The week 0 response curve for *C. vulgaris* showed no apparent lag phase, with exponential growth followed by transition towards the stationary phase of growth. During the following 22 weeks at +4 °C, the experimental cultures of *C. vulgaris* showed a gradual change in time distribution with an apparently longer duration of the exponential growth phase and the emergence of a lag phase (Figure 4.32a). Growth curves for *C. vulgaris* at -20 °C differ significantly in comparison with the initial (week 0) growth response. It is not clear, however, whether the cultures were showing a prolonged lag phase or the rate of exponentially growing cultures changed dramatically (Figure 4.32b).

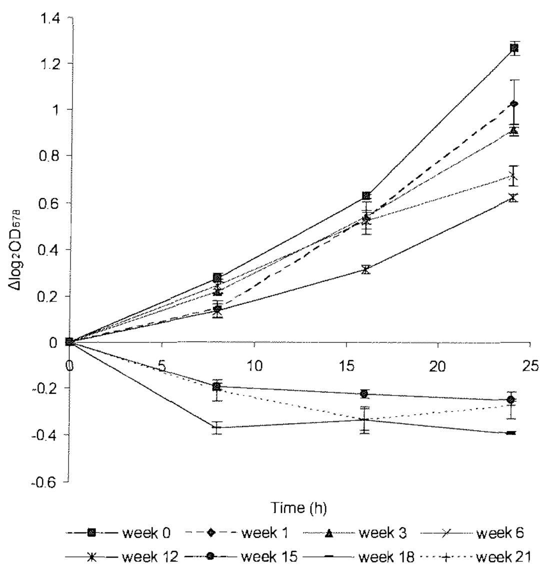
The growth response curve for *S. subspicatus* at week 0 showed an extended lag phase. In the following period the growth response curves appeared to be of two types (Figure 4.32c). In the first (weeks 1-12), *S. subspicatus* group 1 showed an increase in OD_{678}. In the second (weeks 14-22), experimental batches of *S. subspicatus* showed no increase in OD_{678}, and also a decrease in turbidity. This is believed to be due to a degradation of photosynthetic apparatus (Luder *et al.* 2002), when pigment from damaged cells is degraded in the medium. Similar changes in turbidity were obtained for *S. subspicatus* group 2 from week 1 onwards (Figure 4.32d). This supports the idea that unlike *C. vulgaris*, which appears to have some adaptation mechanisms allowing survival of cold and dark conditions, and even of freezing in at least part of the population, cells of *S. subspicatus* are damaged by even relatively short-term exposure to cold and dark and are completely disrupted by freezing.



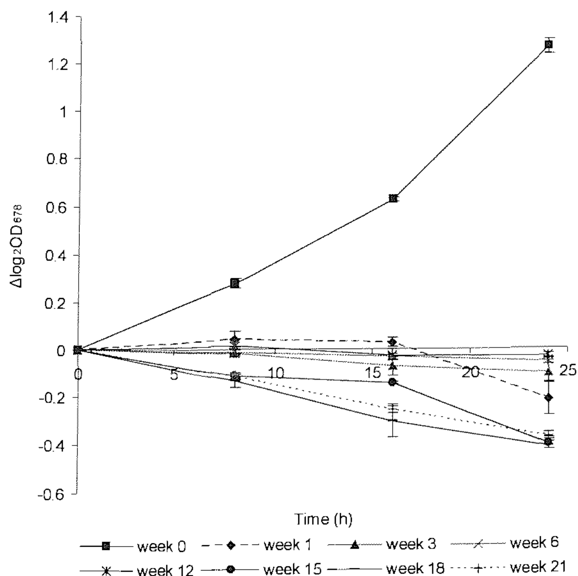
a) *C. vulgaris* group 1



b) *C. vulgaris* group 2



c) *S. subspicatus* group 1

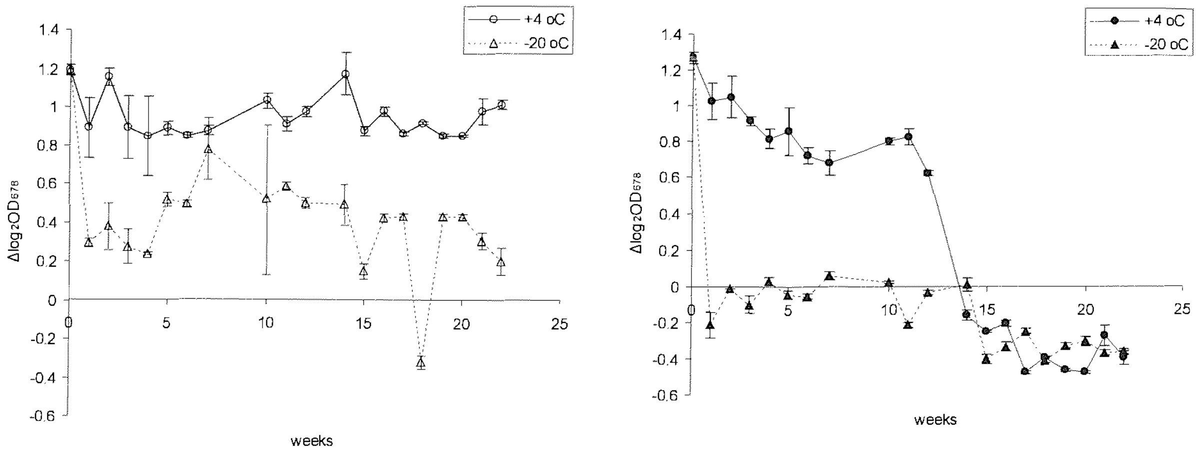


d) *S. subspicatus* group 2

Figure 4.32 Growth response curves for *C. vulgaris* group1 (a) group 2 (b), and *S. subspicatus* group 1 (c) group 2 (d) after storage periods of 0, 1, 3, 6, 12, 15, 18, and 20 weeks. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

Figure 4.33 shows the viability of cultures expressed as specific growth (h^{-1}) after 24 hours of incubation. It can be seen that after periods in complete darkness at low temperatures both species showed alterations in growth response. *C. vulgaris* stored at 4 °C showed a fairly uniform growth response on revival over the 22 week period, averaging about 79% of the week zero value. When stored at -20 °C the response was

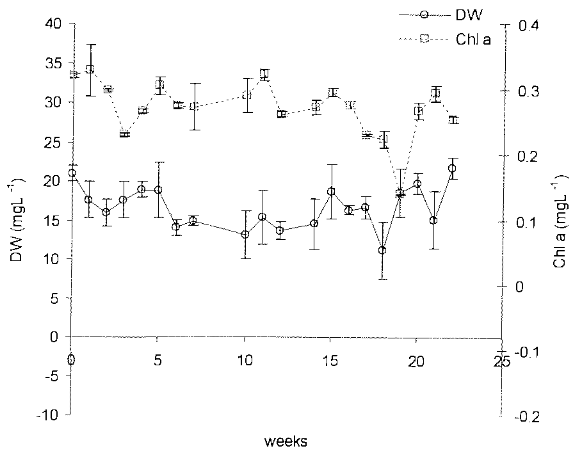
also fairly uniform but the growth recovery was significantly reduced at 31% of the week 0 value. *S. Subspicatus* at 4 °C showed a gradual loss in recoverable growth response over the first 12 weeks followed by a complete loss of response. At -20 °C there was no indication of any potential response.



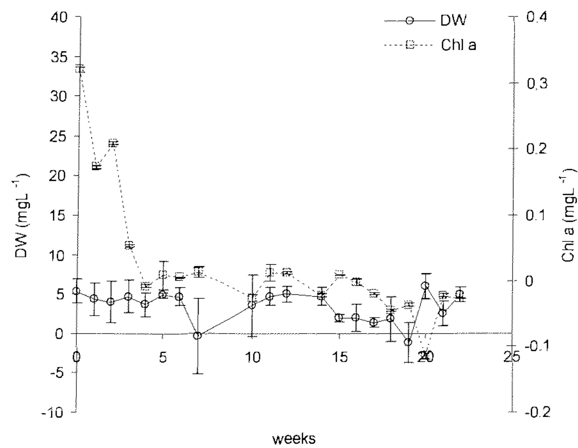
a) *C. vulgaris* group1 and 2 b) *S. subspicatus* group 1 and 2

Figure 4.33 Viability of *C. vulgaris* group1 and 2 (a) and *S. subspicatus* group 1 and 2 (b) expressed as specific growth (h^{-1}) after 24 hours of incubation. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

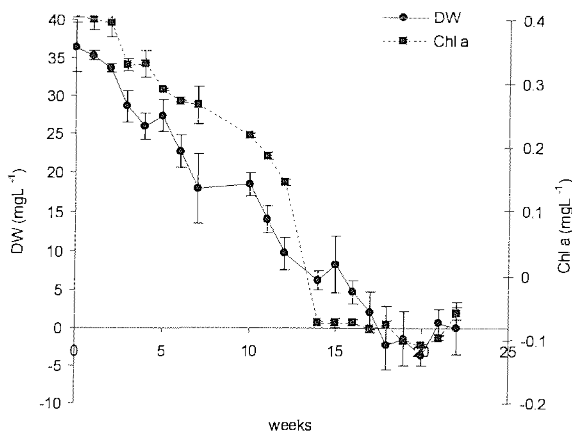
Dry weight and chl **a** concentrations of the cultures were measured directly before and after growth resumption. As seen in Figure 4.34, the increase in algal dry weight and chl **a** is generally consistent with the pattern of cell viability (Figure 4.32) and growth response curves (Figure 4.33) for each of the experimental groups: *C. vulgaris* shows a very slight decline for chl **a** and little or no change for dry weight at +4 °C, with an immediate fall in dry weight and a slightly less rapid one in chlorophyll a at -20 °C, whereas *S. subspicatus* at +4 °C shows a steep decline for dry weight and for chl **a** up to 12 weeks, and a complete lack of response at -20 °C.



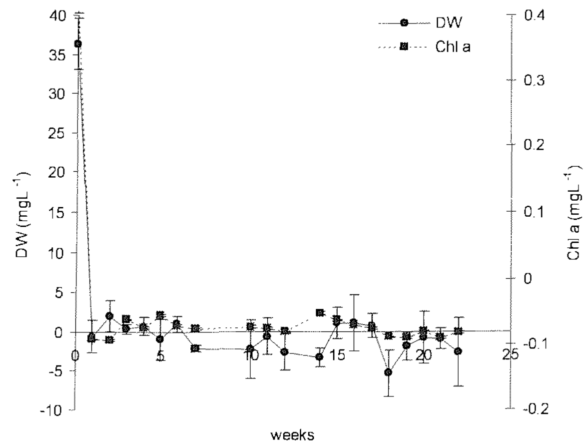
a) *C. vulgaris* group 1



b) *C. vulgaris* group 2



c) *S. subspicatus* group 1



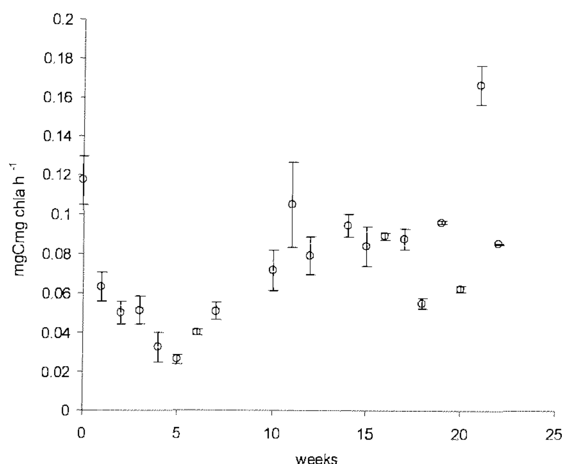
d) *S. subspicatus* group 2

Figure 4.34 Changes in dry weight and chlorophyll a concentrations of *C. vulgaris* group1 (a), group 2 (b) and *S. subspicatus* group 1 (c), group 2 (d) expressed in mg l^{-1} after 24 hours of incubation. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

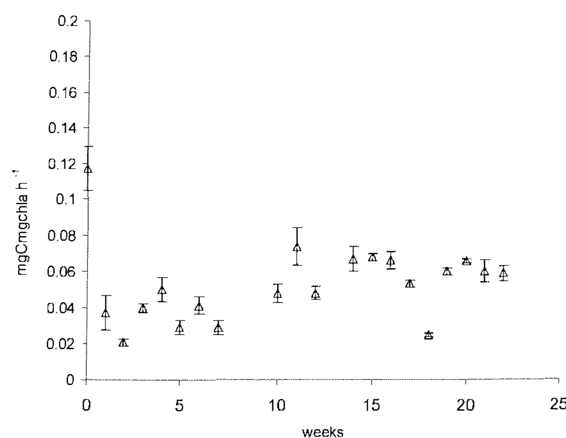
4.6.3 Photosynthetic activity

Figure 4.35a shows the rate of photosynthetic carbon fixation by *C. vulgaris* group 1 cultures. The rate shows a fall from the initial value but no apparently meaningful trend. *C. vulgaris* cultures exposed to -20 ^{o}C showed a sharp initial drop in photosynthetic activity to around 50% of initial values, which then remained fairly stable (Figure 4.35b).

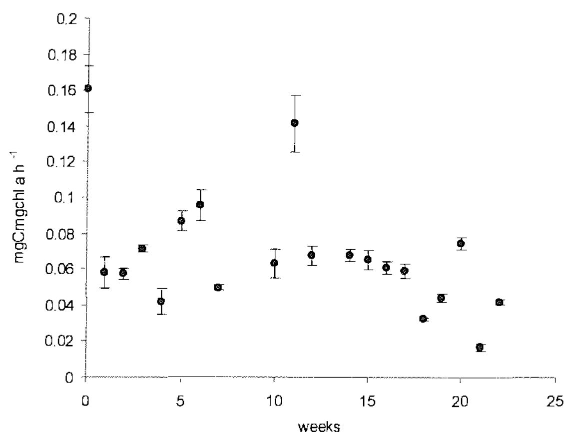
After one week at +4 °C in complete darkness the cultures of *S. subspicatus* exhibited a significant decrease in photosynthetic activity, which remained around 50% of the initial value (Figure 4.35c). Cultures of *S. subspicatus* group 2 were found to be photosynthetically inactive throughout the experimental period.



a) *C. vulgaris* group 1



b) *C. vulgaris* group 2



c) *S. subspicatus* group 1

Figure 4.35 Photosynthesis by *C. vulgaris*, group 1 (a) and 2 (b), and *S. subspicatus*, group 1 (c), expressed as mg C $mg^{-1}h^{-1}$ DW after 24 hours of incubation. Error bars indicate scattering in values among replicates, not shown if smaller than symbol.

4.6.4 Discussion

At 4 °C *C. vulgaris* showed survival of 74% for the whole experimental period of 22 weeks, while *S. subspicatus* showed a higher initial survival of 99% for 8 weeks followed by a rapid decline in the next few weeks to 12%. Dehning & Tizler (1989) measured the survival of *Scenedesmus acuminatus* for periods of 3 months in

complete darkness at 7 °C and 22 °C. They found that 15-25% of cells survived, with better results at 7 °C. It was suggested that improved survival at 7 °C was due to lower metabolic activity. Cell viability decreased as determined by cell numbers and dry weight, and the volume of individual cells doubled. The techniques used differed slightly from those in the present study but the results indicate a significant survival potential especially at lower temperatures.

In addition to testing at 4 °C the two cultures used in the present experiments were also frozen. As discussed, there is evidence that plant tissues including unicellular organisms can be hardened to increase resistance to freezing. A study on *Chlorella ellipsoidea* showed that algal cells became hardy in a manner similar to that of some higher plants (Levitt 1966; Fuchigami *et al.* 1971), where accumulation of ATP and $NADPH_2$ due to a high chl **a** content on growth cessation in autumn is necessary for the development of frost hardiness.. The mechanisms for survival in the current experiments are unknown, but studies on Chlorophyta show a wide diversity in the ability of algae to survive the effects of low temperature. The increased resistance to damage by low temperatures displayed in species isolated from northern regions has also confirmed of influence of native environment on algal durability. For example, *Chlorella* sp. isolated from the Antarctic were used in repeated freezing and thawing experiments by Holm-Hansen (1963), suggesting that this species showed the highest resistance from eight tested. Of the two species studied in the current work only *C. vulgaris* showed resistance to freezing. *S. subspicatus* group 2 showed a little or no survival, despite the hardening procedure applied. The survival of both species after cooling probably reflects increased hardiness of the vegetative cells, and not necessarily the presence of special reproductive structures. Not *Chlorella* nor Scenedesmus species are not known to posses any special structures or reproductive adaptations (Hatano *et al.* 1976).

The data from this experiment indicate that some alterations occur within the cells of *S. subspicatus* during extended storage at +4 °C in complete darkness, which caused a gradual decrease in cell viability up to 12-14 weeks. This decline in viability could be due to many factors, but the following three explanations are possible. Firstly, it is possible that the cells lose essential metabolites by diffusion, as low temperatures are known to increase the permeability of the membrane to solutes (Greiff 1960).

Secondly, the cell metabolic reactions may continue at rates sufficient to cause the observed loss in viability (Proom 1951), as complete cessation of metabolic activity requires temperatures below –100 °C (Meryman 1960). Finally the conditions chosen to test the capacity of algal cells to resume growth may not have been optimum for *S. subspicatus*. The last alternative requires further investigation, as only a limited amount of literature on currently exists on this subject.

Chapter 5 GENERAL DISCUSSION AND CONCLUSIONS

5.1 General discussion

The two species of algae showed similar maximum carbon-specific growth rates within the ranges expected for Chlorophyceae and similar levels of carbon limitation, indicating a high affinity for inorganic carbon and the ability to utilise it to very low levels even at low temperatures. These findings support the use of algal ponds in single annual discharge mode in cold climates such as Canada and Alaska where in autumn the residual carbon levels are low and temperature and light intensity are falling. According to the annual cycle of these ponds, in spring the utilisation of accumulated organic carbon is likely to lead to a substantial release of bacterially-generated CO_2; in late autumn the organic nutrients will have been depleted over the summer period and carbon for algal uptake is likely to be available mainly as a result of the carbon dioxide - bicarbonate equilibrium established from the interaction between the ponds and the atmosphere. For the two species in the current study there is some indication of differences in affinity between CO_2 and bicarbonate, based on the ratio of K_m/V_{max} which was higher for *S. subspicatus* than for *C. vulgaris*. This type of difference may give certain algae an advantage in a pond environment in spring when CO_2 is likely to be abundant, whereas the ability of others to assimilate CO_2 rapidly at lower concentrations may be an advantage in autumn.

Both nitrogen and phosphorus appeared to be taken up in excess of immediate growth requirements and the findings of the research support the theory of active uptake moderated by both light and temperature. This makes kinetic experiments based on nitrate and phosphate limitation difficult to carry out, and neither the Monod nor Michaelis-Menten type of model fits the data well due to the non growth-related uptake. The influence of temperature and light may however be important in cold climate pond operation and design, where there is likely to be an accumulation of nutrients over the winter period. This may be coupled with release of nutrients from benthic sediments in the spring turnover.

Temperature stress is one of the most important factors which may influence nutrient uptake since the phase transition of lipids, the conformation of macromolecules, and the kinetics of physiochemical reactions are all profoundly affected by it.

It is interesting that the optimum temperature for growth of both cultures at light intensities higher than 30 $\mu molm^{-2}s^{-1}$ was 15 ^{o}C and was different from findings on nitrate (20 ^{o}C) and phosphate (not found) uptakes. Such differences were also reported for *Chlorella pyreidosa* (Shelef *et al.* 1970) and *Codium fragile* (Hanisak & Harlin 1978). These differences may be explained by the fact that optimum temperatures for enzyme reactions are not the same as those for growth (Innis & Ingraham 1978). The uncoupling between growth and nutrient uptake may be of ecological significance, because the two different temperature optima would effectively widen the temperature range for survival. Thus, at temperatures optimal for growth, the increased rate of nutrient uptake would expeditiously increase cell quota which is required for growth at low temperature.

There are abundant evidences in literature that phosphorus is accumulated by algal cell in excess of its immediate requirements ("luxury consumption"). The effect of luxury consumption underlines the importance of the nutrient to the cells as it plays particularly important role in cellular processes, which involve energy transfer and in nuclear acid synthesis. Mesple at al (1995) demonstrated that amount of phosphorus in phytoplankton is always greater than the phosphorus precipitated (from 26 to 39% of total phosphorus input leaves the pond in form of particulate phosphate, while P removal by precipitation was 16-21% from the total input).

The ability to accumulate these nutrients is likely to reduce them to growth-limiting levels even when the growth limiting factor may be light limitation due to algal self-shading. Nutrients locked up in algal cells are eventually released, and in a cold climate pond system there is substantial evidence to show that this occurs in winter and early spring. The degree and timescale of recycling of nutrients in a real pond system is unknown on a micro-scale, but results from this and other work in batch culture suggest that this may be quite rapid, even over a period of hours. What is clear is that nutrient uptake is both rapid and efficient over a wide range of temperatures and light intensities, which is one of the primary reasons allowing WSPs to operate at low residual nutrient concentrations in the final effluent.

The two species studied exhibited some difference in nutrient uptake rates and this may be a significant factor in determining the population structure at different nutrient concentrations as related to seasonal cycles. *S. subspicatus* showed higher uptake rates than *C. vulgaris* for both nitrate and phosphorus and might therefore be expected to out-compete *C. vulgaris* when these are limited. Organisms like *S. subspicatus* may become dominant in the population during the summer period but would be unlikely to dominate in the early spring when nutrients are abundant.

The competitive abilities of different species of algae are not determined solely by the acquisition of nutrient resources. The composition of the planktonic algal flora of ponds for domestic and animal waste treatments may be subject to drastic changes due to presence of a zooplanktonic taxon. For instance, a collapse of established Scenedesmus population was coinciding with the appearance of rotifers in different geographical locations (Lincoln *et al.* 1983).

Energy is also allocated to maintenance, reproduction, mitigating environmental stress, and to replacing biomass lost due to physical disturbance and herbivory. Changes in photosynthetic rate in both species occurred rapidly in response to variations in the external light and temperature regimes. *S. subspicatus* appeared to have the ability to adjust the concentration of chl a present in its cells in relation to the growth light intensity, giving it a mechanism to adapt to low and changing light intensities that might be found in a pond environment. This supports previous work which has also shown this ability in a variety of species (Fogg, 1955; Riper et al, 1956;Grumbach et al., 1960; Kirk, 1994).

The results indicate that algae can survive, photosynthesise and grow at very low light intensities and temperatures, although oxygen production rates in the winter period will be very low, especially at high latitudes where daylight hours are limited. The two species showed different types of behaviour in response to changes in these conditions. *C. vulgaris* was able to grow more vigorously than *S. subspicatus* at low temperatures and light intensities, but at temperatures above 15 °C the growth rate of *S. subspicatus* continued to rise while that of *C. vulgaris* showed no further increase. In a natural pond environment this may mean that *C. vulgaris* is able to appear earlier and compete more effectively in early spring but may lose its advantage as the temperature and light intensity increases in to the summer period. The current work is

limited with respect to the variety of species that are likely to be present in a WSP but serves to illustrate how pond ecology and thus performance might be influenced by these environmental factors and growth responses of individual species.

The different responses of the two species to dark and cold conditions are again indicative of the range of responses that may occur across a wider population, and have significant implications for over-winter survival and recovery in spring. In particular this is likely to influence the population present from which the early spring blooms may form. A well-designed WSP is unlikely to freeze completely and there is potential for survival in water close to 0 °C; there is also potential for survival within the frozen ice layer. The experimental results indicated that survival in cold water and ice are both possible but are likely to show species differentiation. While light and temperature are particularly difficult environmental parameters to control, it is clear that species can survive in sufficient numbers to provide an inoculum for the spring bloom.

The period of gradually falling temperatures in a WSP at the onset of winter provides a natural hardening process similar to that used to increase the resistance of cells to damage by freezing: the results showed that at least some species are likely to benefit from this. Further work needs to be carried out to look at the types of organisms that survive in cold climate WSPs and the types of mechanisms that may be utilised to facilities this.

5.2 Summary of general conclusions

1) Good relationships were found between dry weight, chl a and cell count with OD_{678} for both *S. subspicatus* and *C. vulgaris*. These were generally independent of the conditions under which the cultures were grown, apart from chl a concentrations in *S. subspicatus* which appeared to increase with decreasing light intensity.

2) Bicarbonate was found to be a suitable carbon source for both species and maximum growth rates of around 0.07 h^{-1} were calculated using Monod kinetics. Maximum growth rates were achieved at concentrations of around 10 mmol C l^{-1}. At low bicarbonate concentrations atmospheric carbon dioxide was used as a carbon source.

3) Inorganic carbon concentration had little or no effect on the rate of photosynthetic oxygen production, which was similar for *S. subspicatus* and *C. vulgaris* at about 0.2 mmol O_2 mg^{-1} h^{-1} chl a.

4) Nitrate uptake rates were rapid, fairly constant and independent of the initial concentration of inorganic carbon in the medium. Below 0.11 mmol N l^{-1} nitrate is likely to be growth limiting. Nitrate uptake became increasingly light dependent with increasing temperature.

5) Phosphate was taken up from the medium in excess of growth requirements leaving very low basal levels in solution. The luxury uptake of phosphate made it difficult to establish the growth-limiting concentration, possibly due to internal recycling of the nutrient even over short periods of time.

6) At a given concentration of nitrate in the medium, the uptake rate for inorganic carbon, nitrogen and phosphorus of *S. subspicatus* was greater than that of *C.vulgaris*.

7) Both light and temperature affected the growth rates of the two species. Both species reached maximum light-specific growth rates at 47.0 µmol m^{-2} s^{-1} at all temperatures tested. Below 15 °C *C. vulgaris* showed higher growth rates than *S. subspicatus*, but this was reversed at 20 °C.

8) There is a general increase in respiration rates with increased temperature and light intensity. Rates of photosynthesis and carbon fixation increased up to 15°C at which point they showed maximum activity. Photosynthesis was observed even at very low light intensities. At high light intensities carbon fixation is not necessarily related to growth, as the highest rates of carbon fixation were found at irradiance levels above 78.3 µmol m^{-2} s^{-1} in *S.subspicatus*.

9) Both *C. vulgaris* and *S. subspicatus* were tolerant to storage at 4 °C in complete darkness over significant periods of time, although *S. subspicatus* showed a significant decrease in viability at time periods over 3 months. On exposure to -20 °C *S. subspicatus* showed no survival despite a hardening procedure while *C. vulgaris* showed a consistent survival rate of about 12%. Both species showed a change in growth response on revival from cold conditions, with the appearance of a lag phase and/or extended exponential phase.

10) Overall the results indicate that *C. vulgaris* is better adapted to growth at low temperature and light intensities, but may be out-competed by *S. subspicatus* in warmer brighter conditions. Cells of *S. subspicatus* are damaged by even relatively short-term exposure to cold and dark and are completely disrupted by freezing, while *C. vulgaris* appears to have some adaptation mechanisms allowing survival of cold and dark conditions, and even of freezing in at least part of the population. In practical terms this means that in the natural environment *C. vulgaris* may appear earlier and compete more effectively in early spring but may lose its advantage as the temperatures and light intensity increases in to the summer period.

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APPENDIX A: TABULATED DATA ON ALGAL GROWTH

Species	μ (h^{-1})	Growth conditions	Literature source
Chlorella ellipsoidea	0.008	25°C saturating light, synthetic medium	(Hoogenhant & Amesz 1965)
Chlorella pyrenoidosa	 0.001 0.002 0.005-0.006 0.016	continuous saturating light, planktonic strain 10°C 20°C 25°C 39°C	(Fogg 1966)
Chlorella sp.	0.028	20°C NO_3, marine, batch culture	(Wheeler *et al.* 1974)
Scenedesmus obliquus	0.019	25°C saturating light, synthetic medium	(Hoogenhant & Amesz 1965)
	μ_{max} (h^{-1})		
Chlorella spp.	0.0542-0.0875	Mesotrophic/Hypereutrophic, Vernal period	(Seip & Reynolds 1995)
Chlorella ellipsoidea	0.050 0.131	15°C 25°C	(Di Toro *et al.* 1971)
Chlorella ellipsoidea	0.131	25°C	(Tamiya *et al.* 1964)
Chlorella pyrenoidosa	0.008	35°C N limited, NO_3, continuous light: 0.05cal cm^{-2} min^{-1}	(Shelef *et al.* 1972)
Chlorella pyrenoidosa	0.008	35°C, NO_3, light: 0.05cal$cm^{-2}min^{-1}$	(Nyholm 1977)

Chlorella pyrenoidosa	0.0083 0.0460 0.1000 0.1625	10°C 15°C 20°C 25°C	(Sorokin & Krauss 1962)
Chlorella pyrenoidosa	0.0083 0.0460 0.1000 0.0817-0.0896	high-temperature strain 10°C 15°C 20°C 25°C	(Di Toro *et al.* 1971)
Chlorella pyrenoidosa	0.0813-0.0892	25°C, P limiting, continuous culture, freshwater	(Jørgensen *et al.* 1991)
Chlorella pyrenoidosa	0.0817	25°C	(Jørgensen *et al.* 1991)
Chlorella pyrenoidosa	0.0896	25°C	(Sorokin & Krauss 1958)
Chlorella pyrenoidosa	0.0570 0.0770 0.1642 0.1775	NH_4 limiting, continuous culture, freshwater 19°C 28.5°C 35°C, 39.2°C	(Jørgensen *et al.* 1991)
Chlorella pyrenoidosa	0.0600 0.0783 0.0925 0.1800 0.2354	NO_3 limiting, continuous culture, freshwater 19°C 25°C 28.5°C 35.5°C 39.2°C	(Jørgensen *et al.* 1991)
Chlorella vulgaris	0.0750	25°C	(Sorokin & Krauss 1958)
Chlorella vulgaris	0.0750	25°C	(Di Toro *et al.* 1971)
Scenedesmus	0.0625	Eutrophic/Hypereutrophic, Vernal period	(Seip & Reynolds 1995)

Scenedesmus obliquus	0.0633	25°C	(Sorokin & Krauss 1958)
Scenedesmus obliquus	0.0633	25°C	(Di Toro *et al.* 1971)
Scenedesmus quadricauda	0.0842	25°C	(Jørgensen *et al.* 1991)
Scenedesmus quadricauda	0.0842	25°C	(Di Toro *et al.* 1971)

APPENDIX B: CALCULATION EXAMPLES

B.1 Calculations of specific growth rates from OD_{678} readings

Specific growth rate coefficients, μ, of the experimental algal suspensions were calculated using the modification of the Optical Density (OD) method proposed by Sorokin (1973).

Increase in OD for a cell suspension was determined at defined time intervals (h) with Scanning Spectrophotometer (Cecil 3000 series) as follows:

$$OD = \log(\frac{I}{I_0}) \tag{B.1}$$

where I is the light transmission of the sample; I_0 is the light transmission of the blank and OD is optical density readings on λ = 678 nm.

Table B.1 presents a set of OD_{678} readings for *C. vulgaris* culture grown at the presence of 11.1 mmol l^{-1} of inorganic carbon in the medium (section 4.2 "Effects of Carbon enrichment on algal growth") as an example of detailed calculations of the μ. The steps of the calculation are described as it is shown in columns:

Column 1 is the experimental time at which the OD_{678} readings were taken.

The OD_{678} readings from the assay culture as it changed in the course of the growth experiment are given in Column 2.

The conversion of the data into $\log_{10} OD_{678}$ is shown in Column 3.

Column 4 represents a conversion procedure to log with base 2 by multiplying the data from column 3 by 3.32 (for algal cultures, it is more convenient to use base 2 logarithms, which yields increase of algal biomass per unit time). Also, possible

errors connected to the use of negative logarithms were avoided by using an arbitrary figure 10 to transform all negative logarithms into positive ones.

To facilitate plotting of the data, the initial optical density of the cell suspension and, therefore, the $\log_2 OD_{678} + 10$ at zero time was treated as equal to zero and the increases in $\log_2 OD_{678} + 10$ at the subsequent readings were obtained by subtracting from the values of $\log_2 OD_{678} + 10$ at each of the readings the values of $\log_2 OD_{678} + 10$ at zero time. These Δ values in Column 5 actually represent the number of doublings of OD_{678} at indicated time intervals from the beginning of observations (zero time).

Table B.1 Experimental set of data (OD_{678}) for *C. vulgaris* grown with 11.1 mmol l^{-1} of inorganic carbon in the medium.

Time (hrs)	OD_{678}	$\log_{10}OD_{678}$	$\log_2 OD_{678}$ +10	$\Delta \log_2 OD_{678}$
0	0.065	-1.19044029	6.0477	0.0000
8	0.095	-1.02227639	6.6060	0.5583
16	0.129	-0.88941029	7.0472	0.9994
24	0.184	-0.73439612	7.5618	1.5141
32	0.281	-0.55180916	8.1680	2.1203
40	0.349	-0.45758957	8.4808	2.4331
48	0.396	-0.40230481	8.6643	2.6166
56	0.420	-0.37709552	8.7480	2.7003
64	0.413	-0.38440061	8.7238	2.6761
72	0.382	-0.41793664	8.6125	2.5647

Further determination of the growth rate during exponential phase was done by fitting the experimental data into line of the best fitting (Figure B1). The linear fit was chosen by selection the best fitting on the basis of linear regression coefficient. In this example the exponential growth phase was observed between 0 and 32-th hours of the experimental time. The gradient of the linear slope was assumed to be a specific growth rate coefficient, μ (h^{-1}) and in this example was equal to 0.0649 h^{-1} at R^2=0.9975. Note that data for lag phase would be not taken into account in case of its presence.

To facilitate the calculation of maximum growth rate of algae, the "Solver" option in Microsoft Excell was used.

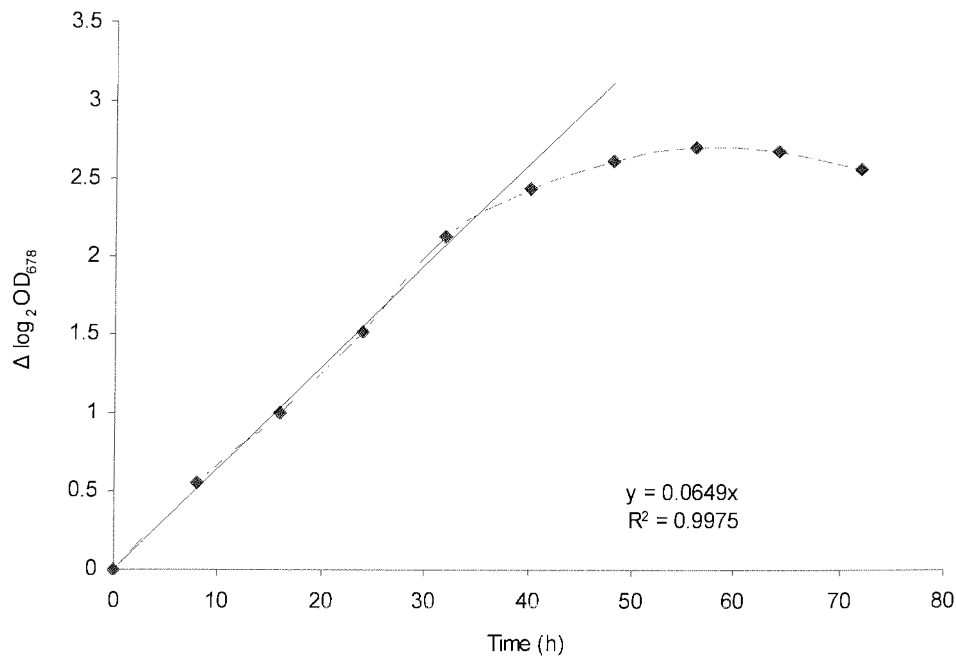

Figure B.1 Plotting calculated values of $\Delta \log_2 OD_{678}$ *versus* experimental time for *C. vulgaris* grown with 11.1 mmolC l^{-1} in the medium. Dashed line is overall experimental data and, the solid line is the bets fitting for experimental data.

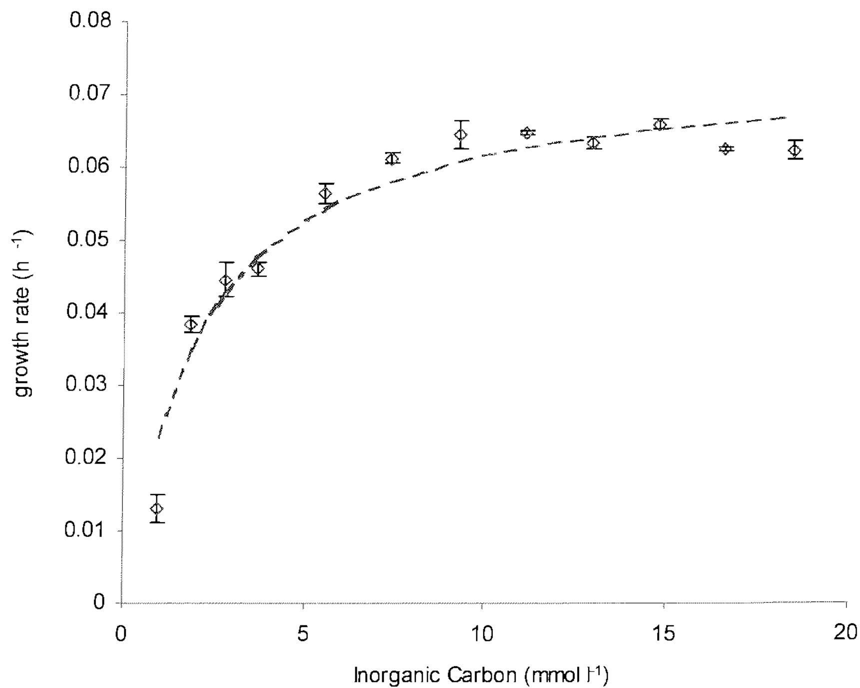

Figure B.2 Effect of inorganic carbon concentration on the specific growth rates of *C.vulgaris*. Dashed lines represent the Monod fitting to the experimental results.

B.2 Calculations of oxygen concentrations from flask's headspace samples

Since the experimental aggregate contained two distinctive environments, the calculations of oxygen production by algal cells were divided into two parts:

1) gas phase (headspace of the reactor), and

2) liquid phase (algal cells in the growth medium).

Calculations of gas phase oxygen concentration: Measured values of oxygen (%) produced during every 8 hours of the experiment were corrected on the amount of control measurements of atmospheric O_2 contamination. The corrected values of O_2 expressed as percentage were converted into volumetric values (ml) by solving the ratio, which equates the total volume of headspace to 100% and percentage of O_2 as unknown volume. Secondly, by applying the ideal gas property to the known volume of O_2 (assuming that 1 M of O_2 occupies 24l of volume).

Calculation of oxygen concentration in liquid phase: Calculation of oxygen concentration in liquid portion of the reactor was initiated by assuming that the measured amount of oxygen in gas phase is equal to the amount of oxygen into liquid phase (equilibrium conditions in reactor). Thus, corrected percentage of O_2 was translated into actual O_2 concentration in liquid phase of the reactor by translating it into O_2 ml (taken a known liquid volume of medium as 100% and solving the ratio) and applying Henry's constants for different temperatures (numerical values were taken from Nazaroff and Alvarez-Cohen 2001) to convert into actual concentration of oxygen in the liquid phase of the flask. Finally, the correction on dissolved oxygen initially presented in medium (before flasks were sealed) was made.

Correction on oxygen contamination: At the beginning of an experiment (time zero) a concentration of oxygen is out of balance due to N_2 filling of flask's headspace. Thus, an equilibrium partial pressure of oxygen in the gas phase is:

$$C_{O2} = K_{H,O2} P_{O2}$$

where C_{O2} is the measured dissolved oxygen concentration in the liquid phase.

Applying a material balance principle to oxygen the second equation can be written as:

$$C_{O2}V_w + \frac{P_t V_a}{RT} = \text{amount of } O_2 \text{ in moles}$$

where $K_{H,O2}$ is the Henry's law constant for oxygen; V_w and V_a are the water and air volumes in the flask; R is the universal gas constant; and T is the temperature (K).

B.3 Calculations of photosynthetic carbon uptake from ^{14}C experiment

The average value of experimental data was calculated from triplicates (in dpm) for "light" variants for each reading followed by calculation of the average values for "dark" and "blank". The use of the average values from all "dark" and all "blank" readings could be justified by the fact that photosynthetic carbon uptake cannot be perform without light or by lifeless algal cells and, therefore values for "dark" and "blank" variants are an indication of magnitude of passive diffusion of inorganic carbon into cell intraspace.

The true photosynthetic ^{14}C uptake, (dpm) was calculated by subtracting the average of "dark" readings from the average "light " for every hour.

Photosynthesis (mg C m^3 h^{-1}) was calculated using the following equation:

$$P = \frac{R_S \times W}{R_T \times N}$$

R_T is the total activity (dpm) of ^{14}C-bicarbonate added; N is the intervals between readings (1 hour); R_S is the true photosynthetic 14C uptake (dpm); W is the weight of DIC present (mg C/m^3).

OD_{678} readings for the parallel "cold" variants were converted into values of chl a concentrations by applying the empirical conversion factor (see section 4.1). Normalisation of photosynthetic carbon assimilation to chlorophyll concentrations of the experimental suspension was made.

Appendix C: Experimental Data

Table C. 1 Rates of inorganic nitrogen uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic carbon in the medium.

Carbon (mmol l^{-1})	Rates of inorganic nitrogen uptake (μmol N $mg^{-1}h^{-1}$ DW)	
	S. subspicatus	*C. vulgaris*
5.55	0.185	0.131
7.40	-	0.145
9.25	0.17	0.199
11.10	0.154	0.209
12.94	0.178	0.191
14.79	0.172	0.179
16.64	0.163	0.134
18.49	0.166	0.118

Table C. 2 Average rates (n=3) of inorganic phosphorus uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic carbon in the medium.

Carbon (mmol l^{-1})	Rates of inorganic phosphorus uptake (μmol P $mg^{-1}h^{-1}$ DW)	
	S. subspicatus	*C. vulgaris*
5.55	0.013	0.015
7.40	-	0.009
9.25	0.011	0.006
11.10	0.017	0.007
12.94	0.010	0.009
14.79	0.007	0.015
16.64	0.011	0.003
18.49	0.011	0.004

Table C. 3 Average (n=3) rates of inorganic carbon uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic nitrogen in the medium.

Nitrate (mmol l^{-1})	Rates of inorganic carbon uptake (μmol C $mg^{-1}h^{-1}$DW)	
	S. subspicatus	*C. vulgaris*
0	0.443	1.985
0.06	0.397	0.930
0.11	0.442	1.115
0.23	0.497	0.466
0.46	0.384	0.656
0.91	0.631	0.915
1.14	0.526	0.628
2.28	0.664	0.362
3.42	0.491	1.007
4.56	0.459	0.827

Table C. 4 Average rates (n=3) of nitrate uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic nitrogen in the medium.

Nitrate (mmol l^{-1})	Rates of nitrate uptake (μmol N $mg^{-1}h^{-1}$DW)	
	S. subspicatus	*C. vulgaris*
0.06	0.015	0.035
0.11	0.050	0.073
0.23	0.063	0.093
0.46	0.086	0.140
0.91	0.147	0.174
1.14	0.149	0.194
2.28	0.164	0.219
3.42	0.245	0.198
4.56	0.213	0.247

Table C. 5 Average rates (n=3) of phosphate uptake by *S. subspicatus* and *C. vulgaris* cultures grown with different initial concentrations of inorganic nitrogen in the medium.

Nitrate (mmol N l^{-1})	Rates of inorganic phosphorus uptake (μmol P $mg^{-1}h^{-1}$ DW)	
	S. subspicatus	*C. vulgaris*
0	0.014	0
0.06	0.014	0.012
0.11	0.019	0.018
0.23	0.018	0.016
0.46	0.012	0.017
0.91	0.012	0.018
1.14	0.014	0.018
2.28	0.011	0.017
3.42	0.012	0.016
4.56	0.013	0.010

Table C. 6 Average rates (n=3) of inorganic carbon uptake by *S. subspicatus* and *C. vulgaris* cultures grown at different initial concentrations of inorganic phosphorus in the medium.

Phosphorus (mol l^{-1})	Rates of inorganic carbon uptake (μmol C $mg^{-1}h^{-1}$DW)	
	S. subspicatus	*C. vulgaris*
0	1.587	1.196
0.05	1.578	0.538
0.10	1.815	1.069
0.19	1.732	1.749
0.39	1.760	1.763
0.58	1.127	1.872

Table C. 7 Average rates (n=3) of nitrate uptake by *S. subspicatus* and *C. vulgaris* cultures grown at different initial concentrations of inorganic phosphorus in the medium.

Phosphorus (mmol l^{-1})	Rates of Inorganic Nitrogen uptake (μmolNmg^{-1}h^{-1}DW)	
	S. subspicatus	*C. vulgaris*
0	0.102	0.168
0.05	0.264	0.101
0.10	0.299	0.102
0.19	0.254	0.099
0.39	0.322	0.133
0.58	0.213	0.121

Table C. 8 Average rates (n=3) of inorganic phosphorus uptake by *S. subspicatus* and *C. vulgaris* cultures grown at different initial concentrations of inorganic phosphorus in the medium.

Phosphorus (mmol l^{-1})	Rates of inorganic phosphorus uptake (μmol P mg^{-1}h^{-1}DW)	
	S. subspicatus	*C. vulgaris*
0	-	-
0.05	0.003	0.021
0.10	0.005	0.037
0.19	0.006	0.051
0.39	0.029	0.042
0.58	0.048	0.037

Calibration experiments: data for *Chlorella vulgaris*

Calibration N.1

Variant 1 (Temperature: 20°C; Light: 7.8 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.946	0.001	255.0	10.0	2.243	0.183	2.6E+04	1.7E+03
0.5	0.501	0.001	125.0	5.8	1.192	0.061	1.2E+04	9.6E+02
0.25	0.260	0.001	62.5	2.9	0.492	0.027	7.9E+03	1.3E+03
0.125	0.136	0.000	30.6	1.3	0.237	0.015	4.0E+03	8.9E+02
0.0625	0.074	0.001	15.3	0.6	0.125	0.007	2.7E+03	4.5E+02
0.03125	0.043	0.000	7.7	0.3	0.063	0.002	1.3E+03	2.7E+02

Variant 2 (Temperature: 20°C; Light: 15.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.895	0.001	240.0	16.3	1.890	0.064	3.0E+04	1.5E+03
0.5	0.471	0.000	117.5	5.0	1.013	0.039	1.7E+04	1.0E+03
0.25	0.241	0.002	61.3	2.5	0.521	0.007	9.3E+03	7.6E+02
0.125	0.126	0.001	28.8	1.4	0.255	0.008	5.5E+03	7.3E+02
0.0625	0.067	0.001	14.1	0.6	0.124	0.012	2.8E+03	2.6E+02

Variant 3 (Temperature: 20°C; Light: 31.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.651	0.001	180.0	0.0	1.452	0.045	2.1E+04	9.1E+02
0.5	0.341	0.001	85.0	5.8	0.709	0.027	1.1E+04	1.0E+03
0.25	0.173	0.001	41.3	2.5	0.414	0.019	5.7E+03	6.9E+02
0.125	0.089	0.001	21.9	1.3	0.194	0.008	2.9E+03	6.1E+02
0.0625	0.046	0.000	10.6	0.7	0.097	0.003	1.2E+03	3.1E+02
0.03125	0.024	0.001	5.0	0.0	0.049	0.002	5.3E+02	2.5E+02

Variant 4 (Temperature: 20°C; Light: 47.0 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.624	0.001	160.0	16.3	1.526	0.064	2.4E+04	1.1E+03
0.5	0.331	0.001	82.5	9.6	0.969	0.043	1.3E+04	1.8E+03
0.25	0.170	0.001	45.0	2.0	-	-	6.3E+03	1.0E+03
0.125	0.087	0.001	25.0	0.0	0.240	0.012	2.9E+03	6.4E+02
0.0625	0.044	0.001	10.6	0.7	0.112	0.006	1.5E+03	3.9E+02
0.03125	0.023	0.000	5.8	0.6	0.059	0.001	8.1E+02	5.0E+02

Variant 5 (Temperature: 20°C; Light: 62.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.816	0.000	240.0	0.0	1.830	0.072	2.3E+04	9.2E+02
0.5	0.427	0.002	117.5	9.6	0.888	0.041	1.3E+04	7.0E+02
0.25	0.215	0.001	57.5	2.9	0.536	0.014	5.9E+03	6.9E+02
0.125	0.106	0.000	28.1	1.3	0.338	0.006	4.1E+03	9.8E+02
0.0625	0.054	0.001	14.4	0.7	0.170	0.007	1.8E+03	4.3E+02
0.03125	0.028	0.000	7.0	0.3	0.084	0.004	1.5E+03	6.4E+02

Variant 6 (Temperature: 20°C; Light: 78.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.570	0.001	133.3	11.5	1.382	0.205	1.7E+04	9.3E+02
0.5	0.285	0.001	63.3	5.8	0.692	0.115	9.0E+03	1.6E+03
0.25	0.143	0.000	30.0	0.0	0.412	0.024	5.2E+03	8.0E+02
0.125	0.071	0.000	15.0	0.0	0.246	0.010	2.8E+03	5.4E+02
0.0625	0.035	0.000	8.3	1.4	0.116	0.008	1.7E+03	3.4E+02
0.03125	0.018	0.001	4.0	0.4	0.059	0.002	8.8E+02	3.0E+02

SD-standard deviation

Calibration experiments: data for *Chlorella vulgaris*

Calibration N.2

Variant 1 (Temperature: 20°C; Light: 7.8 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.503	0.002	142.5	5.0	0.982	0.053	1.7E+04	7.3E+02
0.5	0.261	0.001	66.2	4.8	0.485	0.036	7.8E+03	4.8E+02
0.25	0.131	0.001	33.1	1.2	0.256	0.032	4.2E+03	4.8E+02
0.125	0.068	0.001	16.9	0.7	0.129	0.007	2.4E+03	3.2E+02
0.0625	0.033	0.000	8.0	0.6	0.068	0.006	1.1E+03	1.9E+02

Variant 2 (Temperature: 20°C; Light: 15.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.507	0.001	145.0	12.9	1.141	0.073	1.7E+04	4.8E+02
0.5	0.262	0.001	66.3	2.5	0.572	0.053	8.2E+03	5.5E+02
0.25	0.133	0.001	33.8	1.4	0.287	0.045	4.7E+03	3.5E+02
0.125	0.065	0.000	17.8	1.2	0.115	0.005	2.2E+03	4.9E+02
0.0625	0.031	0.000	8.8	0.0	0.077	0.005	1.2E+03	2.7E+02

Variant 3 (Temperature: 20°C; Light: 31.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.509	0.001	152.5	5.0	1.015	0.126	1.8E+04	8.0E+02
0.5	0.264	0.000	75.0	0.0	0.555	0.155	8.2E+03	5.8E+02
0.25	0.135	0.001	36.3	1.4	0.312	0.013	4.5E+03	3.6E+02
0.125	0.070	0.000	18.1	0.7	0.139	0.008	2.5E+03	2.5E+02
0.0625	0.035	0.000	9.2	0.3	0.058	0.008	1.4E+03	2.7E+02

Variant 4 (Temperature: 20°C; Light: 47.0 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.509	0.001	155.0	5.8	1.094	0.122	1.8E+04	5.7E+02
0.5	0.264	0.001	73.8	6.3	0.637	0.031	1.0E+04	1.1E+03
0.25	0.140	0.001	37.5	0.0	0.341	0.025	7.4E+03	1.5E+03
0.125	0.069	0.000	18.4	0.6	-	-	3.3E+03	6.6E+02
0.0625	0.037	0.001	9.4	0.0	0.081	0.009	1.9E+03	2.7E+02

Variant 5 (Temperature: 20°C; Light: 62.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.515	0.001	122.5	5.0	1.191	0.072	1.5E+04	6.7E+02
0.5	0.268	0.000	60.0	0.0	0.577	0.089	8.2E+03	5.1E+02
0.25	0.137	0.001	30.6	1.2	0.308	0.057	4.7E+03	3.6E+02
0.125	0.069	0.001	14.7	0.6	0.152	0.004	2.8E+03	3.5E+02
0.0625	0.035	0.001	7.8	0.4	0.077	0.009	1.7E+03	3.6E+02

Variant 6 (Temperature: 20°C; Light: 78.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.524	0.001	147.5	5.0	1.279	0.064	1.7E+04	7.3E+02
0.5	0.273	0.000	77.5	2.9	0.561	0.057	1.0E+04	3.4E+02
0.25	0.142	0.001	38.1	1.3	0.297	0.013	5.8E+03	8.2E+02
0.125	0.075	0.000	18.8	1.0	0.134	0.012	3.3E+03	4.6E+02
0.0625	0.038	0.000	8.6	0.3	0.090	0.014	1.6E+03	3.5E+02

SD-standard deviation

Calibration experiments: data for *Scenedesmus subspicatus*

Calibration N.1

Variant 1 (Temperature: 20°C; Light: 7.8 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.633	0.002	195.0	10.0	3.548	0.040	1.1E+04	1.3E+03
0.5	0.315	0.001	97.5	5.0	1.616	0.068	5.3E+03	4.3E+02
0.25	0.160	0.001	47.5	2.9	1.146	0.054	3.7E+03	6.6E+02
0.125	0.083	0.001	23.1	1.3	0.573	0.023	2.0E+03	4.3E+02
0.0625	0.042	0.000	12.2	0.6	0.263	0.013	1.1E+03	2.7E+02
0.03125	0.023	0.000	6.0	0.4	0.167	0.009	5.6E+02	2.6E+02

Variant 2 (Temperature: 20°C; Light: 15.7 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.340	0.002	105.0	5.8	2.007	0.068	1.1E+04	6.7E+02
0.5	0.174	0.001	50.0	0.0	0.862	0.031	5.4E+03	7.9E+02
0.25	0.090	0.000	25.0	0.0	0.537	0.026	4.0E+03	6.7E+02
0.125	0.046	0.001	11.9	0.7	0.207	0.008	2.9E+03	3.7E+02
0.0625	0.023	0.001	5.9	0.4	0.136	0.005	1.6E+03	3.3E+02

Variant 3 (Temperature: 20°C; Light: 31.3 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.824	0.001	275.0	10.0	2.823	0.062	1.4E+04	1.1E+03
0.5	0.428	0.002	132.5	9.6	1.747	0.082	7.6E+03	4.8E+02
0.25	0.215	0.001	66.2	2.5	0.706	0.033	4.4E+03	5.7E+02
0.125	0.109	0.001	35.0	0.0	0.372	0.014	2.2E+03	4.2E+02
0.0625	0.055	0.000	17.2	0.6	0.204	0.010	9.7E+02	2.8E+02
0.03125	0.029	0.001	8.7	0.6	0.137	0.001	5.6E+02	2.6E+02

Variant 4 (Temperature: 20°C; Light: 47.0 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.739	0.002	245.0	10.0	2.768	0.083	1.2E+04	1.8E+03
0.5	0.377	0.001	117.5	5.0	1.514	0.077	6.5E+03	6.6E+02
0.25	0.189	0.001	57.5	2.9	0.931	0.044	3.8E+03	8.3E+02
0.125	0.093	0.001	28.8	1.4	0.408	0.020	1.9E+03	2.2E+02
0.0625	0.049	0.000	14.1	0.6	0.203	0.009	9.7E+02	2.1E+02
0.03125	0.022	0.001	6.7	0.3	0.119	0.005	3.9E+02	2.0E+02

Variant 5 (Temperature: 20°C; Light: 62.7 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.400	0.003	155.0	10.0	1.222	0.044	8.5E+03	7.0E+02
0.5	0.199	0.002	72.5	5.0	0.608	0.022	4.8E+03	8.5E+02
0.25	0.100	0.001	31.3	2.5	0.351	0.017	2.6E+03	6.8E+02
0.125	0.051	0.001	16.3	1.4	0.179	0.006	1.8E+03	3.8E+02
0.0625	0.026	0.000	8.1	0.7	0.095	0.003	6.4E+02	2.8E+02
0.03125	0.013	0.001	3.9	0.3	0.048	0.001	3.1E+02	1.2E+02

Variant 6 (Temperature: 20°C; Light: 78.3 µmol m⁻²s⁻¹)

dilution	OD_{678} ±SD		DW (mg l⁻¹) ±SD		chl a (mg l⁻¹) ±SD		cells (l⁻¹) ±SD	
1	0.510	0.001	160.0	5.8	1.129	0.073	7.3E+03	6.1E+02
0.5	0.260	0.001	76.7	6.3	0.570	0.053	4.3E+03	6.4E+02
0.25	0.123	0.001	44.4	0.0	0.243	0.045	2.4E+03	4.4E+02
0.125	0.066	0.000	20.8	0.6	0.116	0.005	1.1E+03	4.0E+02
0.0625	0.031	0.000	11.3	0.5	0.057	0.009	5.3E+02	2.5E+02
0.03125	0.017	0.000	5.0	0.0	0.029	0.005	2.8E+02	8.8E+01

SD-standard deviation

Calibration experiments: data for *Scenedesmus subspicatus*

Calibration N.2

Variant 1 (Temperature: 20°C; Light: 7.8 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.931	0.002	330.0	11.5	4.023	0.250	1.9E+04	8.4E+02
0.5	0.473	0.001	157.5	5.0	2.259	0.104	9.9E+03	5.3E+02
0.25	0.242	0.001	76.2	8.5	1.172	0.110	5.3E+03	5.0E+02
0.125	0.123	0.001	37.5	2.0	0.583	0.072	2.2E+03	5.0E+02
0.0625	0.064	0.000	20.0	1.0	0.287	0.017	1.1E+03	1.9E+02
0.03125	0.033	0.001	9.7	0.4	0.144	0.012	5.9E+02	1.9E+02

Variant 2 (Temperature: 20°C; Light: 15.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.934	0.000	355.0	10.0	3.966	0.161	1.7E+04	1.3E+03
0.5	0.483	0.001	192.5	5.0	2.067	0.131	9.8E+03	4.6E+02
0.25	0.246	0.000	93.8	2.5	1.033	0.034	5.1E+03	4.6E+02
0.125	0.124	0.001	46.3	1.4	0.530	0.018	2.6E+03	3.3E+02
0.0625	0.062	0.001	23.1	1.3	0.275	0.015	1.3E+03	2.7E+02
0.03125	0.031	0.000	11.9	0.0	0.130	0.005	6.6E+02	1.9E+02

Variant 3 (Temperature: 20°C; Light: 31.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.929	0.001	355.0	25.2	3.907	0.115	1.9E+04	8.5E+02
0.5	0.486	0.001	202.5	5.0	2.117	0.029	1.1E+04	8.3E+02
0.25	0.248	0.001	96.3	2.5	1.077	0.043	6.0E+03	9.4E+02
0.125	0.130	0.002	48.8	1.4	0.557	0.032	3.0E+03	4.4E+02
0.0625	0.059	0.001	22.5	0.0	0.262	0.015	1.5E+03	3.9E+02
0.03125	0.035	0.001	12.0	0.3	0.138	0.005	7.8E+02	1.6E+02

Variant 4 (Temperature: 20°C; Light: 47.0 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.928	0.002	345.0	10.0	4.334	0.062	2.1E+04	1.5E+03
0.5	0.477	0.001	175.0	5.8	2.175	0.105	8.8E+03	9.4E+02
0.25	0.240	0.001	87.5	5.0	1.083	0.035	5.0E+03	6.5E+02
0.125	0.120	0.001	42.5	0.0	0.535	0.048	2.9E+03	8.9E+02
0.0625	0.060	0.001	20.9	0.6	0.254	0.011	1.6E+03	4.0E+02
0.03125	0.030	0.001	10.6	0.5	0.128	0.002	8.8E+02	3.0E+02

Variant 5 (Temperature: 20°C; Light: 62.7 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.929	0.001	335.0	10.0	3.383	0.387	2.0E+04	6.6E+02
0.5	0.480	0.001	182.5	74.1	1.669	0.037	9.8E+03	4.2E+02
0.25	0.242	0.001	95.0	4.1	0.952	0.030	5.1E+03	9.2E+02
0.125	0.122	0.001	47.5	2.0	0.378	0.019	3.2E+03	1.0E+03
0.0625	0.065	0.001	21.9	0.7	0.271	0.019	2.6E+03	7.2E+02
0.03125	0.033	0.001	11.4	0.3	0.085	0.008	1.5E+03	2.5E+02

Variant 6 (Temperature: 20°C; Light: 78.3 µmol $m^{-2}s^{-1}$)

dilution	OD_{678} ±SD		DW (mg l^{-1}) ±SD		chl a (mg l^{-1}) ±SD		cells (l^{-1}) ±SD	
1	0.932	0.001	365.0	10.0	3.096	0.128	1.6E+04	7.3E+02
0.5	0.482	0.001	185.0	10.0	1.940	0.129	8.5E+03	8.7E+02
0.25	0.245	0.000	92.5	6.5	0.939	0.013	4.2E+03	4.8E+02
0.125	0.124	0.001	45.0	2.9	0.503	0.008	2.0E+03	2.8E+02
0.0625	0.061	0.000	22.8	1.2	0.279	0.008	1.2E+03	2.1E+02
0.03125	0.028	0.001	12.0	0.3	0.098	0.009	8.8E+02	3.0E+02

SD-standard deviation

Carbon enrichment experiment: Data for *Scenedesmus subspicatus*

Concentration of carbon source (HCO_3^-) in experimental variants for *S.subspicatus*

variant	C (mmol l^{-1})	Exponential phase* (h)	
1	0.925	0	36
2	1.849	0	36
3	2.774	0	36
4	3.698	0	36
5	5.548	0	40
7	9.246	0	40
8	11.095	0	32
9	12.944	0	40
10	14.793	0	40
11	16.643	0	40
12	18.492	0	40

Variant 1 (0.925 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.066	0.000	-	-	-	-	-	-
12	0.092	0.002	-	-	-	-	-	-
24	0.103	0.001	-	-	-	-	-	-
36	0.121	0.000	-	-	-	-	-	-
48	0.122	0.001	-	-	-	-	-	-
60	0.129	0.000	-	-	-	-	-	-
72	0.130	0.000	-	-	-	-	-	-
84	0.135	0.002	-	-	-	-	-	-
96	0.127	0.001	-	-	-	-	-	-
108	0.128	0.001	-	-	-	-	-	-
120	0.127	0.001	-	-	-	-	-	-

Variant 2 (1.849 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.067	0.000	-	-	-	-	-	-
12	0.096	0.001	-	-	-	-	-	-
24	0.119	0.001	-	-	-	-	-	-
36	0.162	0.000	-	-	-	-	-	-
48	0.167	0.001	-	-	-	-	-	-
60	0.173	0.001	-	-	-	-	-	-
72	0.171	0.000	-	-	-	-	-	-
84	0.179	0.001	-	-	-	-	-	-
96	0.178	0.001	-	-	-	-	-	-
108	0.179	0.001	-	-	-	-	-	-
120	0.170	0.001	-	-	-	-	-	-

Variant 3 (2.773 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.066	0.000	-	-	-	-	-	-
12	0.103	0.001	-	-	-	-	-	-
24	0.132	0.001	-	-	-	-	-	-
36	0.191	0.000	-	-	-	-	-	-
48	0.212	0.002	-	-	-	-	-	-
60	0.222	0.001	-	-	-	-	-	-
72	0.224	0.001	-	-	-	-	-	-
84	0.219	0.000	-	-	-	-	-	-
96	0.220	0.001	-	-	-	-	-	-
108	0.220	0.001	-	-	-	-	-	-
120	0.229	0.004	-	-	-	-	-	-

* the time (h) of the onset and the end of the exponential phase of growth.
SD-standard deviation

Carbon enrichment experiment: Data for *Scenedesmus subspicatus*

Variant 4 (3.698 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.067	0.000	-	-	-	-	-	-
12	0.105	0.001	-	-	-	-	-	-
24	0.142	0.000	-	-	-	-	-	-
36	0.220	0.001	-	-	-	-	-	-
48	0.237	0.001	-	-	-	-	-	-
60	0.259	0.001	-	-	-	-	-	-
72	0.262	0.001	-	-	-	-	-	-
84	0.262	0.001	-	-	-	-	-	-
96	0.264	0.000	-	-	-	-	-	-
108	0.264	0.001	-	-	-	-	-	-
120	0.261	0.001	-	-	-	-	-	-

Variant 5 (5.548 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.083	0.000	61.103	0.838	16.735	0.059	4.464	1.042
8	0.107	0.001	61.553	0.748	15.744	0.545	4.136	0.687
16	0.133	0.003	62.453	0.577	14.826	0.327	4.464	0.456
24	0.175	0.002	59.190	1.226	11.565	0.399	4.136	0.240
32	0.251	0.002	50.883	0.690	8.454	0.230	3.654	0.438
40	0.326	0.004	34.857	1.644	6.753	0.045	3.070	0.395
48	0.366	0.001	17.323	0.760	4.872	0.662	2.509	0.866
56	0.393	0.003	15.403	0.961	4.276	0.384	2.086	0.330
64	0.402	0.002	14.260	1.216	3.331	0.392	2.250	0.137
72	0.403	0.002	12.620	1.018	2.899	0.392	1.669	0.104

Variant 7 (9.246 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.083	0.000	109.000	0.265	16.684	0.090	4.820	0.310
8	0.119	0.002	96.677	7.579	17.135	0.091	4.842	0.376
16	0.157	0.005	96.683	7.636	15.691	0.312	4.494	0.400
24	0.224	0.002	90.600	1.832	14.946	0.046	4.349	0.818
32	0.314	0.001	82.113	1.320	11.474	0.064	4.514	0.028
40	0.409	0.005	61.713	0.725	9.443	0.180	3.827	0.485
48	0.480	0.004	37.130	0.981	6.689	1.353	3.036	1.851
56	0.505	0.004	27.647	0.331	5.046	0.536	3.387	2.340
64	0.521	0.002	24.700	0.574	3.813	0.279	2.500	0.521
72	0.521	0.002	21.667	1.850	3.225	0.046	2.338	0.400

Variant 8 (11.095 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.083	0.000	122.690	0.059	13.856	0.002	2.374	0.063
8	0.110	0.002	120.630	1.476	14.713	0.066	8.050	0.464
16	0.139	0.010	112.660	2.760	11.049	2.620	11.214	0.126
24	0.189	0.016	105.650	1.594	9.687	0.056	7.777	0.326
32	0.265	0.002	91.983	2.720	5.377	0.015	5.703	0.153
40	0.335	0.012	82.697	1.965	3.035	0.033	5.703	0.045
48	0.374	0.015	72.867	2.027	2.180	0.030	5.658	0.046
56	0.399	0.011	61.580	1.679	1.825	0.010	2.908	0.191
64	0.408	0.014	49.360	1.493	1.608	0.025	2.234	0.007
72	0.412	0.017	43.870	1.077	1.498	0.005	2.329	0.077

SD-standard deviation

Carbon enrichment experiment: Data for *Scenedesmus subspicatus*

Variant 9 (12.944 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.085	0.000	147.667	3.612	15.598	0.306	5.041	0.060
8	0.118	0.001	139.233	7.477	13.976	0.074	4.331	0.150
16	0.164	0.006	141.133	3.937	12.102	0.066	5.041	0.205
24	0.249	0.002	122.567	8.911	8.010	0.090	3.960	0.122
32	0.353	0.001	114.933	1.137	4.059	0.064	5.319	0.422
40	0.455	0.008	91.427	0.344	1.484	0.016	3.650	0.025
48	0.554	0.004	62.900	0.531	0.634	0.034	2.441	0.186
56	0.588	0.005	50.047	1.199	0.466	0.036	2.873	0.083
64	0.601	0.003	46.210	0.701	0.397	0.001	2.533	0.208
72	0.600	0.003	42.220	0.805	0.450	0.002	2.853	0.026

Variant 10 (14.793 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.084	0.000	185.200	1.015	15.473	0.054	4.118	0.506
8	0.119	0.001	169.750	0.919	13.621	0.051	3.971	0.217
16	0.164	0.007	147.000	1.442	11.919	0.029	4.118	0.245
24	0.248	0.002	147.000	1.442	7.490	0.062	3.781	0.074
32	0.363	0.001	131.567	1.168	3.996	0.029	3.019	0.188
40	0.468	0.008	108.400	2.227	1.408	0.034	3.407	0.036
48	0.559	0.006	77.983	2.721	0.159	0.019	2.494	0.225
56	0.602	0.001	62.673	0.315	0.074	0.009	1.990	0.016
64	0.628	0.006	54.740	0.541	0.137	0.007	2.533	0.445
72	0.642	0.002	54.073	1.284	0.113	0.012	3.217	0.050

Variant 11 (16.643 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.083	0.000	205.433	1.060	14.908	0.016	4.391	0.169
8	0.122	0.001	192.350	4.738	14.037	0.020	4.001	0.494
16	0.167	0.006	178.467	2.515	10.664	0.027	3.609	0.078
24	0.243	0.003	168.533	1.484	6.794	0.738	3.538	0.272
32	0.357	0.003	154.033	1.501	3.904	0.010	2.665	0.303
40	0.471	0.008	134.033	0.850	1.477	0.042	2.610	0.039
48	0.569	0.003	99.627	0.676	0.094	0.031	2.357	0.343
56	0.620	0.003	82.830	1.850	0.042	0.005	1.861	0.154
64	0.650	0.005	73.633	0.913	0.067	0.023	1.936	0.217
72	0.666	0.002	66.143	1.040	0.129	0.010	1.717	0.018

Variant 12 (18.492 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.083	0.000	217.600	0.424	14.480	0.027	3.587	0.185
8	0.120	0.001	160.333	0.971	13.413	0.008	3.572	0.089
16	0.152	0.006	201.433	1.504	10.939	0.048	3.207	0.070
24	0.226	0.002	193.800	0.265	7.549	0.021	2.974	0.178
32	0.322	0.003	179.900	0.300	4.210	0.002	2.362	0.570
40	0.441	0.011	161.233	1.137	1.696	0.019	1.856	0.129
48	0.557	0.004	125.933	0.751	0.023	0.011	1.405	0.147
56	0.619	0.006	98.730	1.588	0.048	0.010	1.583	0.021
64	0.649	0.003	82.750	1.412	0.170	0.009	0.826	0.106
72	0.663	0.001	69.830	0.880	0.059	0.010	1.523	0.033

SD-standard deviation

Carbon enrichment experiment: Data for *Scenedesmus subspicatus*

Scenedesmus subspicatus: Oxygen production (mg l^{-1}) ±SD

Time (h)	Variant 5		Variant 7		Variant 8		Variant 9	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	43.735	3.285	43.735	0.155	54.175	5.585	39.533	7.494
16	100.922	13.783	100.922	2.379	107.863	13.552	103.828	13.328
24	130.362	16.192	130.362	15.183	163.250	22.827	140.105	21.849
32	178.631	31.640	178.631	1.993	232.525	16.025	189.566	28.240
40	230.811	42.462	230.811	8.196	314.058	31.143	254.611	33.877
48	292.028	40.885	292.028	29.484	406.649	21.960	310.852	48.442
56	326.486	54.259	326.486	4.984	449.738	17.632	384.799	41.326
64	350.331	23.188	350.331	5.348	479.083	37.709	417.236	12.321
72	361.500	60.078	361.500	5.518	502.296	39.537	431.605	99.066

Time (h)	Variant 10		Variant 11		Variant 12	
0	0.000	0.000	0.000	0.000	0.000	0.000
8	65.737	3.936	54.114	13.106	31.281	3.190
16	115.488	23.070	103.936	10.396	73.249	7.964
24	186.366	36.225	169.763	21.980	119.017	10.924
32	276.394	50.252	247.267	44.221	181.819	29.457
40	373.468	82.064	329.988	63.181	259.199	25.783
48	483.428	109.434	444.491	84.356	359.194	27.221
56	553.951	126.862	497.579	113.769	419.697	120.799
64	607.960	154.105	529.830	112.715	458.106	120.879
72	642.326	162.816	552.972	117.639	484.042	127.723

Carbon enrichment experiment: data for *Scenedesmus subspicatus*

Control flasks

	DIC (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	88.433	1.071	123.800	0.361	195.167	5.435
8	88.253	0.647	122.93	1.654	195.92	3.697
16	89.067	2.108	123.77	1.153	197.33	3.046
24	88.030	1.097	123.91	0.835	196.59	1.381
32	87.940	1.857	122.49	1.858	197.63	1.723
40	87.853	1.052	121.99	2.702	197.34	2.997
48	89.070	0.636	124.23	0.866	197.55	2.110
56	86.843	1.139	121.83	1.318	199.08	0.688
64	86.993	1.312	123.08	1.948	196.46	2.564
72	88.977	1.457	124.30	0.683	197.86	2.994

	N (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	14.980	6.188	14.579	0.021	15.740	0.002
8	15.020	2.882	15.079	0.033	15.734	0.012
16	14.908	4.671	13.913	0.213	15.048	0.044
24	15.080	3.188	14.288	0.133	15.436	0.018
32	14.180	6.188	13.913	0.570	15.744	0.041
40	14.568	2.115	14.098	1.325	15.794	0.204
48	12.449	3.188	13.810	0.457	14.834	0.010
56	15.120	5.019	14.513	1.013	15.048	0.804
64	14.350	2.171	14.584	0.020	15.974	1.002
72	13.780	3.015	13.513	0.216	15.361	0.410

	P (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	4.468	1.612	3.277	0.138	5.902	0.047
8	4.084	1.181	3.782	0.008	5.707	0.461
16	3.895	1.006	4.002	1.084	5.802	1.111
24	4.115	2.113	3.128	0.784	5.277	1.230
32	5.015	1.119	3.207	0.881	4.977	0.983
40	4.401	2.212	2.821	0.654	6.111	0.705
48	4.325	0.866	3.267	1.013	6.328	0.112
56	4.499	0.116	3.133	0.876	6.112	1.110
64	5.001	0.207	4.211	1.441	5.547	0.761
72	4.217	1.834	3.282	0.154	5.224	0.030

SD-standard deviation

Carbon enrichment experiment: data for *Chlorella vulgaris*

Concentration of carbon (HCO_3^-) in experimental medium for *Chlorella vulgaris*

variant	C (mmol l^{-1})	Exponential phase* (h)	
1	0.925	8	32
2	1.849	0	32
3	2.774	0	8
4	3.698	0	10
5	5.548	0	24
6	7.397	8	32
7	9.246	0	32
8	11.095	0	32
9	12.944	0	32
10	14.793	0	32
11	16.643	0	40
12	18.492	0	40

Variant 1 (0.925 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.060	0.000	-	-	-	-	-	-
8	0.082	0.001	-	-	-	-	-	-
16	0.089	0.002	-	-	-	-	-	-
24	0.095	0.002	-	-	-	-	-	-
32	0.102	0.003	-	-	-	-	-	-
40	0.104	0.005	-	-	-	-	-	-
48	0.103	0.000	-	-	-	-	-	-
56	0.103	0.000	-	-	-	-	-	-
64	0.101	0.000	-	-	-	-	-	-
72	0.103	0.000	-	-	-	-	-	-
80	0.103	0.000	-	-	-	-	-	-

Variant 2 (1.849 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.050	0.000	-	-	-	-	-	-
8	0.063	0.002	-	-	-	-	-	-
16	0.082	0.002	-	-	-	-	-	-
24	0.093	0.001	-	-	-	-	-	-
32	0.115	0.005	-	-	-	-	-	-
40	0.121	0.006	-	-	-	-	-	-
48	0.123	0.000	-	-	-	-	-	-
56	0.128	0.000	-	-	-	-	-	-
64	0.129	0.000	-	-	-	-	-	-
72	0.136	0.000	-	-	-	-	-	-
80	0.141	0.000	-	-	-	-	-	-

Variant 3 (2.773 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.168	0.002	-	-	-	-	-	-
2	0.181	0.005	-	-	-	-	-	-
4	0.192	0.006	-	-	-	-	-	-
6	0.204	0.005	-	-	-	-	-	-
8	0.214	0.005	-	-	-	-	-	-
10	0.223	0.004	-	-	-	-	-	-
12	0.227	0.005	-	-	-	-	-	-
14	0.229	0.005	-	-	-	-	-	-
16	0.230	0.004	-	-	-	-	-	-

* the period of the exponential growth phase for algal cultures (the onset and the end)
SD-standard deviation

Carbon enrichment experiment: data for *Chlorella vulgaris*

Variant 4 (3.698 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.169	0.000	-	-	-	-	-	-
2	0.181	0.002	-	-	-	-	-	-
4	0.195	0.002	-	-	-	-	-	-
6	0.206	0.001	-	-	-	-	-	-
8	0.217	0.001	-	-	-	-	-	-
10	0.231	0.003	-	-	-	-	-	-
12	0.235	0.005	-	-	-	-	-	-
14	0.237	0.006	-	-	-	-	-	-
16	0.238	0.006	-	-	-	-	-	-

Variant 5 (5.548 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.065	0.000	64.433	0.611	12.613	0.835	3.705	0.111
8	0.087	0.001	61.250	0.325	13.259	0.001	3.705	0.154
16	0.123	0.005	62.790	0.393	12.060	0.027	3.313	0.077
24	0.166	0.002	56.550	0.184	10.860	0.048	3.072	0.192
32	0.200	0.003	53.873	0.542	8.943	0.071	2.908	0.087
40	0.219	0.005	53.063	1.489	8.494	0.504	2.821	0.092
48	0.234	0.005	49.513	1.790	8.379	0.006	2.785	0.107
56	0.241	0.010	49.957	0.724	8.269	0.029	2.976	0.276
64	0.240	0.014	48.063	1.001	8.136	0.012	2.884	0.036
72	0.244	0.015	44.493	0.850	7.723	0.034	2.755	0.365

Variant 6 (7.397 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.065	0.000	88.433	1.071	14.980	6.188	4.468	1.612
8	0.085	0.003	89.580	2.872	14.989	0.066	3.526	0.016
16	0.122	0.000	84.760	1.492	12.244	0.009	3.451	0.227
24	0.171	0.006	79.793	0.766	10.474	0.009	3.334	0.043
32	0.232	0.004	76.750	1.873	7.155	0.029	3.207	0.274
40	0.282	0.005	80.460	1.274	7.060	0.030	3.078	0.018
48	0.310	0.005	62.060	0.979	6.930	0.039	2.841	0.088
56	0.330	0.002	59.777	1.476	6.685	0.027	2.933	0.093
64	0.336	0.001	76.363	0.725	6.704	0.011	2.930	0.231
72	0.332	0.004	69.250	0.876	6.012	0.069	2.663	0.111

Variant 7 (9.246 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.065	0.000	105.933	0.808	14.572	5.052	3.042	1.195
8	0.090	0.003	101.607	1.959	14.465	2.112	3.357	1.139
16	0.127	0.003	100.520	1.180	12.575	0.019	3.338	0.462
24	0.182	0.003	92.873	0.110	11.577	2.028	3.366	0.880
32	0.272	0.010	86.520	1.342	7.343	0.109	2.763	0.016
40	0.339	0.027	82.033	0.535	6.198	0.118	2.578	0.154
48	0.390	0.042	74.433	0.954	5.390	0.039	2.580	0.206
56	0.423	0.053	68.777	0.849	4.419	0.039	2.172	0.208
64	0.426	0.059	68.193	0.159	4.749	0.057	2.352	0.329
72	0.398	0.045	64.470	0.781	4.348	0.127	2.143	0.431

SD-standard deviation

Carbon enrichment experiment: data for *Chlorella vulgaris*

Variant 8 (11.095 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.065	0.000	123.800	0.361	14.579	0.021	3.277	0.138
8	0.094	0.001	123.367	0.351	14.154	0.026	3.293	0.076
16	0.128	0.001	122.433	1.168	13.813	0.008	3.038	0.255
24	0.185	0.001	114.533	0.153	10.904	0.044	2.991	0.230
32	0.279	0.002	102.633	0.666	6.680	1.367	2.846	0.228
40	0.347	0.003	97.163	0.906	6.329	0.034	2.383	0.139
48	0.396	0.001	90.780	0.632	5.409	0.027	2.612	0.246
56	0.417	0.005	89.040	0.185	4.888	0.010	2.270	0.047
64	0.415	0.006	86.733	0.496	4.931	0.055	2.394	0.195
72	0.383	0.007	84.167	1.187	4.463	0.029	2.043	0.251

Variant 9 (12.944 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.065	0.000	154.867	1.365	14.536	0.059	4.103	0.624
8	0.093	0.002	149.700	1.114	14.186	0.108	3.704	0.190
16	0.125	0.002	145.500	1.572	13.136	0.121	3.492	0.139
24	0.180	0.006	137.967	1.050	10.906	0.032	3.942	0.328
32	0.275	0.001	133.700	0.721	7.555	0.010	3.613	0.099
40	0.364	0.006	118.100	1.480	5.963	0.014	3.336	0.160
48	0.429	0.004	110.200	0.721	5.514	0.028	3.342	0.052
56	0.465	0.008	104.800	0.173	4.298	0.011	2.432	0.380
64	0.472	0.011	111.533	8.260	4.117	0.026	2.253	0.331
72	0.433	0.018	104.167	1.528	3.736	0.012	2.160	0.332

Variant 10 (14.793 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.063	0.000	181.500	2.211	15.977	0.001	9.020	0.007
8	0.087	0.000	167.900	2.343	15.429	0.004	8.409	0.038
16	0.125	0.001	167.300	1.905	14.630	0.006	8.985	0.004
24	0.181	0.005	176.133	0.987	13.166	0.025	9.020	0.036
32	0.294	0.007	160.630	0.656	9.263	0.010	8.258	0.039
40	0.394	0.014	156.700	0.557	8.836	0.021	7.463	0.060
48	0.474	0.027	180.267	3.121	7.182	0.011	8.216	0.002
56	0.531	0.030	127.333	0.635	5.980	0.011	7.349	0.256
64	0.544	0.033	118.500	0.900	5.236	0.014	6.063	0.518
72	0.524	0.010	133.233	1.193	4.857	0.014	2.248	0.576

Variant 11 (16.643 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.064	0.000	195.167	5.435	15.740	0.002	5.902	0.047
8	0.086	0.001	196.500	0.608	15.485	0.009	5.529	0.038
16	0.126	0.004	198.167	1.966	14.553	0.005	5.286	0.021
24	0.181	0.004	196.933	0.252	12.973	0.021	5.578	0.007
32	0.251	0.017	167.433	0.862	10.290	0.028	4.186	0.082
40	0.356	0.010	183.733	4.219	8.115	0.017	6.153	0.004
48	0.497	0.027	178.967	2.743	6.043	0.006	4.470	0.004
56	0.579	0.006	177.600	6.986	4.500	0.010	3.659	0.036
64	0.593	0.006	172.033	1.644	2.668	1.772	3.629	3.222
72	0.572	0.018	186.967	2.219	3.525	0.005	3.200	0.013

SD-standard deviation

Carbon enrichment experiment: data for *Chlorella vulgaris*

Variant 12 (18.492 mmol l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.072	0.000	219.217	0.887	15.097	0.031	6.287	0.015
8	0.099	0.003	220.372	0.584	14.245	0.005	6.187	0.017
16	0.135	0.002	222.993	0.173	13.381	0.012	6.424	0.045
24	0.179	0.006	206.650	0.616	10.424	0.013	6.847	0.021
32	0.294	0.025	204.287	0.266	9.817	0.000	5.313	0.032
40	0.434	0.032	200.825	0.907	6.739	0.023	6.300	0.012
48	0.534	0.037	177.042	0.142	8.058	0.016	4.904	0.024
56	0.469	0.191	168.688	2.056	6.959	0.006	2.815	0.022
64	0.471	0.196	175.653	0.360	5.104	0.006	6.208	0.012
72	0.482	0.208	169.867	0.360	3.901	0.006	5.217	0.022

***Chlorella vulgaris*: Oxygen production (mg l^{-1}) ±SD**

Time (h)	Variant 5		Variant 6		Variant 7		Variant 8	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	23.582	11.204	80.442	0.285	41.824	4.312	24.518	9.551
16	34.579	11.638	131.760	3.106	81.685	10.263	44.789	14.707
24	51.603	16.730	158.189	18.424	133.119	18.614	68.938	24.539
32	71.042	19.688	174.991	1.952	178.124	12.276	107.280	37.438
40	76.952	14.157	187.448	6.656	216.624	21.481	136.279	45.388
48	100.074	14.011	199.912	20.184	243.665	13.158	163.749	58.268
56	122.936	32.724	219.980	3.358	272.776	10.694	186.763	76.086
64	124.914	33.251	223.520	3.412	284.074	22.360	199.315	85.612
72	126.940	33.790	227.144	3.467	288.680	22.723	202.546	87.000

Time (h)	Variant 9		Variant 10		Variant 11		Variant 12	
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	31.892	14.666	46.634	11.295	16.613	6.678	30.400	22.752
16	61.424	12.270	90.342	9.037	37.677	11.632	72.714	19.198
24	86.760	16.864	124.835	16.163	65.664	12.593	121.563	24.290
32	124.909	22.710	175.230	31.338	112.798	18.275	161.671	7.759
40	160.773	35.328	217.876	41.715	156.257	15.543	209.254	4.287
48	191.553	43.362	256.740	48.724	247.683	68.307	236.421	33.628
56	219.213	50.203	278.024	63.569	271.666	78.192	247.121	29.703
64	235.302	59.644	291.542	62.022	287.971	75.986	251.098	30.181
72	239.117	60.611	296.269	63.028	292.640	77.218	258.679	42.513

SD-standard deviation

Carbon enrichment experiment: data for *Chlorella vulgaris*

Control flasks

	DIC (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	88.433	1.071	123.800	0.361	195.167	5.435
8	88.253	0.647	122.93	1.654	195.92	3.697
16	89.067	2.108	123.77	1.153	197.33	3.046
24	88.030	1.097	123.91	0.835	196.59	1.381
32	87.940	1.857	122.49	1.858	197.63	1.723
40	87.853	1.052	121.99	2.702	197.34	2.997
48	89.070	0.636	124.23	0.866	197.55	2.110
56	86.843	1.139	121.83	1.318	199.08	0.688
64	86.993	1.312	123.08	1.948	196.46	2.564
72	88.977	1.457	124.30	0.683	197.86	2.994

	N (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	14.980	6.188	14.579	0.021	15.740	0.002
8	15.020	2.882	15.079	0.033	15.734	0.012
16	14.908	4.671	13.913	0.213	15.048	0.044
24	15.080	3.188	14.288	0.133	15.436	0.018
32	14.180	6.188	13.913	0.570	15.744	0.041
40	14.568	2.115	14.098	1.325	15.794	0.204
48	12.449	3.188	13.810	0.457	14.834	0.010
56	15.120	5.019	14.513	1.013	15.048	0.804
64	14.350	2.171	14.584	0.020	15.974	1.002
72	13.780	3.015	13.513	0.216	15.361	0.410

	P (mg l^{-1}) ±SD					
Time (h)	**variant 6**		**variant 8**		**variant 11**	
0	4.468	1.612	3.277	0.138	5.902	0.047
8	4.084	1.181	3.782	0.008	5.707	0.461
16	3.895	1.006	4.002	1.084	5.802	1.111
24	4.115	2.113	3.128	0.784	5.277	1.230
32	5.015	1.119	3.207	0.881	4.977	0.983
40	4.401	2.212	2.821	0.654	6.111	0.705
48	4.325	0.866	3.267	1.013	6.328	0.112
56	4.499	0.116	3.133	0.876	6.112	1.110
64	5.001	0.207	4.211	1.441	5.547	0.761
72	4.217	1.834	3.282	0.154	5.224	0.030

SD-standard deviation

pH_ experiment for *Chlorella vulgaris*

Experimental conditions for *Chlorella vulgaris*

Variant	C (mmol l^{-1})	flasks
1	2.219	unsealed
2	2.219	sealed
3	22.19	unsealed
4	22.19	sealed
5	110.95	unsealed
6	110.95	sealed
7	221.90	unsealed
8	221.90	sealed

	OD_{678} ±SD							
Time (h)	Variant 1		Variant 2		Variant 3		Variant 4	
0	0.087	0.000	0.087	0.000	0.075	0.000	0.075	0.000
8	0.107	0.000	0.101	0.001	0.091	0.001	0.090	0.001
16	0.128	0.002	0.106	0.001	0.113	0.005	0.102	0.003
24	0.170	0.005	0.096	0.006	0.139	0.001	0.107	0.004
32	0.232	0.006	0.092	0.002	0.187	0.002	0.117	0.013
40	0.287	0.004	0.090	0.001	0.247	0.003	0.123	0.012
48	0.336	0.004	0.090	0.002	0.295	0.006	0.133	0.015
56	0.345	0.006	0.090	0.003	0.306	0.010	0.137	0.017
64	0.356	0.009	0.090	0.002	0.319	0.007	0.141	0.017
72	0.361	0.011	0.090	0.001	0.330	0.002	0.144	0.017

	pH ±SD							
Time (h)	Variant 1		Variant 2		Variant 3		Variant 4	
0	7.200	0.000	7.200	0.000	7.400	0.000	7.400	0.000
8	7.517	0.023	8.927	0.067	8.583	0.031	9.397	0.006
16	7.877	0.015	9.450	0.010	8.753	0.050	9.687	0.012
24	8.003	0.055	9.440	0.026	8.783	0.040	9.767	0.012
32	8.487	0.061	9.477	0.025	9.160	0.036	9.907	0.021
40	8.850	0.046	9.523	0.029	9.477	0.040	10.107	0.072
48	9.177	0.087	9.653	0.021	9.787	0.060	10.387	0.125
56	9.210	0.020	9.690	0.020	9.810	0.010	10.410	0.030
64	9.240	0.010	9.690	0.030	9.840	0.020	10.460	0.040
72	9.270	0.010	10.010	0.010	9.860	0.020	10.480	0.010

	OD_{678} ±SD							
Time (h)	Variant 5		Variant 6		Variant 7		Variant 8	
0	0.104	0.000	0.104	0.000	0.103	0.000	0.103	0.000
8	0.134	0.003	0.140	0.003	0.133	0.000	0.137	0.002
16	0.166	0.003	0.167	0.006	0.165	0.002	0.168	0.002
24	0.216	0.005	0.211	0.005	0.213	0.003	0.214	0.003
32	0.289	0.010	0.245	0.002	0.289	0.009	0.274	0.003
40	0.399	0.013	0.260	0.003	0.402	0.011	0.322	0.007
48	0.522	0.009	0.276	0.006	0.542	0.014	0.381	0.010
56	0.660	0.030	0.279	0.007	0.653	0.022	0.422	0.003
64	0.757	0.029	0.302	0.020	0.778	0.016	0.459	0.009
72	0.814	0.004	0.294	0.010	0.843	0.010	0.478	0.013

	pH ±SD							
Time (h)	Variant 5		Variant 6		Variant 7		Variant 8	
0	7.900	0.000	7.900	0.000	7.900	0.000	7.900	0.000
8	9.257	0.012	9.410	0.000	9.320	0.000	9.370	0.010
16	9.280	0.020	9.580	0.010	9.363	0.006	9.463	0.006
24	9.443	0.021	9.823	0.006	9.480	0.000	9.640	0.010
32	9.587	0.015	9.987	0.006	9.577	0.012	9.800	0.010
40	9.700	0.017	10.047	0.012	9.683	0.015	9.900	0.000
48	9.880	0.020	10.107	0.021	9.857	0.032	10.023	0.006
56	10.140	0.046	10.190	0.035	10.077	0.049	10.170	0.010
64	10.380	0.104	10.220	0.044	10.243	0.050	10.247	0.025
72	10.607	0.065	10.277	0.047	10.437	0.055	10.340	0.017

pH_ experiment for *Scenedesmus subspicatus*

Experimental conditions for *Scenedesmus subspicatus*

Variant	C (mmol l^{-1})	flasks
1	2.219	unsealed
2	2.219	sealed
3	22.19	unsealed
4	22.19	sealed
5	110.95	unsealed
6	110.95	sealed
7	221.90	unsealed
8	221.90	sealed

	OD_{678} ±SD							
Time (h)	Variant 1		Variant 2		Variant 3		Variant 4	
0	0.087	0.000	0.087	0.000	0.101	0.000	0.101	0.000
8	0.097	0.006	0.083	0.006	0.114	0.004	0.123	0.002
16	0.117	0.010	0.087	0.002	0.149	0.006	0.139	0.003
24	0.152	0.007	0.087	0.003	0.190	0.004	0.161	0.002
32	0.211	0.004	0.090	0.006	0.249	0.004	0.175	0.005
40	0.262	0.006	0.085	0.003	0.322	0.003	0.183	0.005
48	0.324	0.009	0.089	0.009	0.370	0.015	0.193	0.007
56	0.332	0.007	0.086	0.003	0.378	0.016	0.198	0.008
64	0.342	0.005	0.087	0.004	0.387	0.020	0.205	0.012
72	0.353	0.004	0.087	0.003	0.402	0.017	0.211	0.010

	pH ±SD							
Time (h)	Variant 1		Variant 2		Variant 3		Variant 4	
0	7.200	0.000	7.200	0.000	7.400	0.000	7.400	0.000
8	9.143	0.214	9.667	0.057	9.507	0.133	9.930	0.339
16	10.003	0.150	9.907	0.121	9.973	0.091	10.443	0.783
24	9.987	0.107	9.833	0.110	10.197	0.032	10.540	0.867
32	10.107	0.266	9.810	0.115	10.387	0.091	10.543	0.870
40	10.523	0.015	9.867	0.118	11.027	0.064	10.600	0.918
48	10.743	0.040	10.000	0.132	11.333	0.012	10.690	0.996
56	10.750	0.010	10.020	0.050	11.350	0.010	10.760	0.010
64	10.780	0.020	10.100	0.060	11.370	0.020	10.790	0.010
72	10.800	0.010	10.120	0.020	11.610	0.010	11.010	0.090

	OD_{678} ±SD							
Time (h)	Variant 5		Variant 6		Variant 7		Variant 8	
0	0.101	0.000	0.101	0.000	0.100	0.000	0.100	0.000
8	0.129	0.004	0.129	0.005	0.119	0.005	0.117	0.003
16	0.161	0.009	0.163	0.003	0.153	0.006	0.159	0.003
24	0.206	0.023	0.220	0.005	0.192	0.017	0.197	0.005
32	0.272	0.007	0.281	0.008	0.250	0.027	0.257	0.013
40	0.354	0.007	0.343	0.011	0.326	0.024	0.345	0.010
48	0.440	0.026	0.390	0.018	0.433	0.002	0.429	0.004
56	0.545	0.034	0.424	0.020	0.552	0.013	0.542	0.012
64	0.604	0.040	0.462	0.018	0.632	0.053	0.636	0.052
72	0.691	0.014	0.491	0.022	0.675	0.020	0.689	0.008

	pH ±SD							
Time (h)	Variant 5		Variant 6		Variant 7		Variant 8	
0	7.900	0.000	7.900	0.000	7.900	0.000	7.900	0.000
8	9.360	0.000	9.503	0.006	9.370	0.000	9.410	0.000
16	9.503	0.025	9.773	0.015	9.457	0.012	9.553	0.006
24	9.580	0.017	10.037	0.047	9.573	0.006	9.720	0.010
32	9.677	0.045	10.557	0.059	9.623	0.031	9.867	0.021
40	9.997	0.032	11.000	0.010	9.720	0.010	10.020	0.010
48	10.283	0.051	11.257	0.042	9.847	0.012	10.323	0.029
56	11.013	0.006	11.437	0.015	10.167	0.055	11.073	0.057
64	11.137	0.050	11.483	0.021	10.793	0.142	11.273	0.040
72	11.263	0.090	11.490	0.017	11.123	0.091	11.490	0.046

Nitrogen enrichment experiment: Data for *Chlorella vulgaris*

Concentration of nitrogen source (NO_3^-) in experimental variants for *Chlorella vulgaris*

variant	N (mmol l^{-1})	Exponential phase* (h)	
1	0.000	0	16
2	0.057	8	32
3	0.114	16	32
4	0.228	16	40
5	0.456	16	40
6	0.913	16	40
7	1.141	16	40
8	2.282	16	40
9	3.424	16	32
10	4.565	16	40

Variant 1 (0.000 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.086	0.000	217.700	1.273	-	-	5.988	0
8	0.099	0.001	218.250	1.344	-	-	5.298	0.690
16	0.115	0.001	215.000	0.141	-	-	5.251	0.822
24	0.119	0.001	215.000	0.424	-	-	5.091	0.929
32	0.121	0.002	217.150	0.354	-	-	5.583	0.244
40	0.122	0.001	219.100	0.990	-	-	5.979	0.602
48	0.123	0.000	219.300	1.556	-	-	5.587	0.039
56	0.124	0.001	218.800	0.908	-	-	5.563	0.046
64	0.127	0.001	217.980	0.080	-	-	5.617	0.156
72	0.128	0.002	217.710	0.123	-	-	5.567	0.126

Variant 2 (0.057 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.084	0.000	215.800	0.566	0.799	0.000	5.848	0.000
8	0.098	0.002	217.400	0.990	0.755	0.075	5.588	0.169
16	0.120	0.003	215.750	0.636	0.329	0.077	5.420	0.087
24	0.166	0.003	216.650	0.071	0.272	0.120	5.086	0.327
32	0.200	0.004	217.200	0.141	0.169	0.083	5.281	0.504
40	0.211	0.004	213.450	0.212	0.276	0.138	5.287	0.127
48	0.217	0.005	211.400	0.566	0.176	0.080	4.963	0.195
56	0.216	0.005	199.300	1.273	0.201	0.139	4.766	0.309
64	0.219	0.005	189.000	1.222	0.163	0.055	4.820	0.173
72	0.223	0.004	187.112	0.023	0.147	0.046	4.753	0.232

Variant 3 (0.114 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.085	0.00	218.733	1.514	1.598	0.000	4.384	0.000
8	0.096	0.00	219.650	0.495	1.463	0.113	5.962	0.343
16	0.119	0.00	220.800	0.849	1.111	0.062	5.778	0.231
24	0.170	0.00	217.550	0.778	0.525	0.155	5.211	0.445
32	0.232	0.00	217.050	0.354	0.220	0.140	4.448	1.603
40	0.263	0.00	214.600	1.652	0.120	0.026	4.963	0.080
48	0.273	0.00	215.150	0.354	0.336	0.165	4.910	0.374
56	0.271	0.00	217.300	0.566	0.162	0.101	4.603	1.100
64	0.277	0.00	210.850	0.636	0.326	0.403	4.801	0.709
72	0.284	0.006	208.000	0.230	0.177	0.068	4.813	0.624

SD-standard deviation

* the period of the exponential growth phase for algal cultures (the onset and the end)

Nitrogen enrichment experiment: Data for *Chlorella vulgaris*

Variant 4 (0.228 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.086	0.000	214.300	4.950	3.195	0.000	4.690	0.000
8	0.098	0.002	219.700	1.697	2.915	0.085	5.639	0.035
16	0.120	0.000	216.150	0.778	2.570	0.010	5.692	0.310
24	0.169	0.003	217.000	1.414	1.800	0.081	5.194	0.339
32	0.237	0.009	215.800	1.637	0.629	0.220	5.054	0.372
40	0.305	0.007	219.550	0.212	0.174	0.026	4.957	0.103
48	0.334	0.001	211.450	1.485	0.259	0.180	4.723	0.692
56	0.341	0.005	216.050	0.212	0.241	0.070	4.749	0.248
64	0.359	0.007	197.550	0.778	0.225	0.062	4.771	0.345
72	0.368	0.004	196.998	0.612	0.173	0.035	4.650	0.567

Variant 5 (0.456 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.085	0.000	225.750	0.354	6.391	0.000	5.846	0.000
8	0.100	0.001	224.100	1.414	5.875	0.192	5.582	0.839
16	0.121	0.001	222.350	0.354	5.429	0.135	5.395	0.813
24	0.174	0.002	222.050	0.071	3.984	1.138	5.110	0.816
32	0.251	0.010	222.600	1.131	3.609	0.970	5.211	0.711
40	0.347	0.021	219.550	0.495	1.414	0.333	4.529	0.868
48	0.430	0.025	212.650	0.354	0.346	0.114	4.454	0.359
56	0.472	0.019	212.533	1.474	0.137	0.079	4.400	0.346
64	0.491	0.008	185.400	0.566	0.132	0.003	4.229	0.308
72	0.527	0.020	198.100	1.273	0.244	0.122	3.905	1.124

Variant 6 (0.913 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.085	0.000	227.800	0.141	12.782	0.000	6.516	0.000
8	0.099	0.001	228.000	0.141	12.344	0.620	5.823	0.136
16	0.120	0.001	228.000	1.744	11.742	0.373	5.565	0.339
24	0.173	0.003	227.550	0.778	10.520	0.258	5.273	0.440
32	0.245	0.004	229.750	1.202	8.986	0.045	5.149	0.562
40	0.332	0.013	228.900	0.707	7.294	0.239	4.680	0.434
48	0.415	0.009	213.900	0.566	5.639	0.305	4.914	0.321
56	0.493	0.012	215.600	1.273	4.136	0.544	3.847	1.040
64	0.558	0.029	234.250	0.354	3.444	0.345	3.870	0.457
72	0.619	0.040	178.750	0.354	2.042	0.758	4.076	0.346

Variant 7 (1.141 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.084	0.000	224.400	1.980	15.977	0.000	3.993	0.000
8	0.093	0.001	223.150	0.636	15.952	0.848	5.581	0.179
16	0.112	0.001	227.500	0.424	15.389	0.771	5.632	0.236
24	0.154	0.001	225.600	0.424	13.962	0.821	5.617	0.167
32	0.209	0.008	225.250	1.202	12.872	1.082	5.200	0.509
40	0.265	0.016	223.900	1.414	11.763	1.395	4.921	0.559
48	0.308	0.030	221.900	0.849	10.960	1.891	4.867	0.634
56	0.345	0.042	218.100	0.283	9.895	1.971	4.591	0.891
64	0.380	0.059	206.800	3.536	9.381	2.262	4.123	1.170
72	0.409	0.076	204.150	0.636	8.767	2.175	4.281	0.804

SD-standard deviation

Nitrogen enrichment experiment: Data for *Chlorella vulgaris*

Variant 8 (2.282 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.084	0.000	227.200	0.707	31.954	0.000	5.882	0.000
8	0.098	0.002	225.650	2.192	31.271	0.598	5.526	0.465
16	0.120	0.001	226.400	1.980	30.513	0.572	5.354	0.255
24	0.171	0.001	224.433	1.415	29.684	1.664	6.226	1.512
32	0.237	0.002	228.700	0.990	28.253	1.385	4.755	0.206
40	0.310	0.003	226.400	0.424	26.399	0.426	5.997	1.630
48	0.367	0.002	206.200	0.424	25.427	0.728	4.368	0.701
56	0.407	0.001	211.650	0.778	23.666	0.263	3.260	2.297
64	0.437	0.004	225.500	2.263	23.627	0.227	4.492	0.382
72	0.462	0.007	202.850	0.636	22.963	0.057	4.487	0.346

Variant 9 (3.424 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.084	0.000	222.900	1.273	47.931	0.000	5.831	0.000
8	0.099	0.003	227.650	1.344	47.006	0.819	5.879	0.699
16	0.121	0.002	223.800	0.849	46.320	0.626	4.945	0.769
24	0.167	0.001	228.150	0.354	45.429	0.361	5.875	0.923
32	0.222	0.002	220.800	0.707	44.428	1.001	5.462	0.733
40	0.267	0.002	217.867	6.854	43.326	0.964	4.584	1.088
48	0.297	0.003	221.050	0.919	43.835	0.610	4.587	0.945
56	0.325	0.005	216.700	1.273	41.957	1.100	5.045	0.818
64	0.344	0.008	221.250	0.919	41.725	0.739	4.784	1.032
72	0.353	0.004	209.900	0.424	37.128	4.475	4.407	0.333

Variant 10 (4.565 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.089	0.000	222.000	0.424	63.908	0.000	3.773	0.000
8	0.101	0.002	223.350	1.202	57.260	3.323	5.346	0.193
16	0.125	0.001	222.450	2.333	57.200	3.588	5.415	0.435
24	0.175	0.003	222.800	0.424	56.379	2.970	5.209	0.163
32	0.236	0.005	219.850	0.212	54.833	2.070	4.847	0.860
40	0.310	0.018	217.400	0.424	54.077	2.159	4.862	0.132
48	0.390	0.006	222.250	0.495	52.883	1.567	4.131	0.749
56	0.427	0.046	219.450	1.626	51.562	0.343	4.697	0.161
64	0.467	0.058	220.650	0.071	51.192	0.866	3.898	0.757
72	0.501	0.062	220.700	1.556	50.277	3.143	4.844	1.186

SD-standard deviation

Nitrogen enrichment experiment: Data for *Scenedesmus subspicatus*

Concentration of nitrogen source (NO_3^-) in experimental variants for *Scenedesmus subspicatus*

variant	N (mmol l^{-1})	Exponential phase* (h)	
1	0.000	0	32
2	0.057	0	40
3	0.114	0	32
4	0.228	0	40
5	0.456	0	48
6	0.913	0	48
7	1.141	0	48
8	2.282	0	48
9	3.424	0	48
10	4.565	0	48

Variant 1 (0.000 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.104	0.000	108.200	0.000	-	-	5.938	0.073
8	0.135	0.006	105.750	0.778	-	-	3.824	3.446
16	0.155	0.005	109.450	0.212	-	-	5.829	0.694
24	0.186	0.004	102.700	0.283	-	-	6.062	0.745
32	0.220	0.007	104.500	0.283	-	-	5.633	0.876
40	0.245	0.002	96.485	0.346	-	-	4.919	1.283
48	0.266	0.001	97.765	1.068	-	-	5.138	0.829
56	0.270	0.002	85.345	0.332	-	-	5.155	0.874
64	0.276	0.005	82.450	0.400	-	-	4.920	0.998
72	0.282	0.004	80.980	0.512	-	-	4.740	0.928

Variant 2 (0.057 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.101	0.000	109.050	0.354	0.823	0.042	5.745	0.203
8	0.132	0.004	107.450	0.495	0.293	0.132	7.031	2.405
16	0.157	0.006	110.150	0.919	0.337	0.398	5.802	0.258
24	0.189	0.002	96.340	0.467	0.463	0.139	6.097	0.854
32	0.235	0.006	103.650	0.354	0.201	0.288	5.899	0.781
40	0.284	0.005	102.200	0.000	0.271	0.258	5.020	0.770
48	0.316	0.004	100.355	0.912	0.269	0.302	4.750	0.645
56	0.321	0.001	85.240	0.226	0.379	0.057	5.570	0.996
64	0.326	0.001	83.850	0.450	0.193	0.146	4.923	0.176
72	0.331	0.003	82.100	0.090	0.120	0.080	5.197	0.455

Variant 3 (0.114 mmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.098	0.000	110.250	0.495	1.630	0.055	5.858	0.048
8	0.128	0.011	107.200	0.990	0.679	0.551	5.487	0.505
16	0.158	0.010	103.000	0.424	0.158	0.112	5.998	0.824
24	0.202	0.003	105.100	0.141	0.135	0.074	5.567	0.191
32	0.273	0.008	99.210	0.735	0.124	0.126	4.971	0.263
40	0.329	0.008	98.030	0.042	0.142	0.125	4.805	0.122
48	0.370	0.006	95.150	0.382	0.257	0.117	5.167	0.806
56	0.374	0.006	83.200	0.552	0.318	0.123	4.938	0.472
64	0.381	0.006	80.090	0.320	0.210	0.095	5.130	0.197
72	0.387	0.005	78.910	0.300	0.137	0.095	5.037	0.559

SD-standard deviation

* the period of the exponential growth phase for algal cultures (the onset and the end)

Nitrogen enrichment experiment: Data for *Scenedesmus subspicatus*

Variant 4 (0.228 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.101	0.000	107.950	0.071	3.195	0.000	5.960	0.155
8	0.132	0.006	105.150	0.212	2.867	1.313	5.218	0.893
16	0.151	0.004	104.350	0.778	1.253	1.158	5.583	0.510
24	0.212	0.003	107.050	0.354	0.443	0.566	4.966	0.239
32	0.280	0.002	87.960	0.311	0.065	0.050	4.911	0.176
40	0.356	0.007	88.085	1.181	0.193	0.093	4.491	0.469
48	0.422	0.008	80.380	0.877	0.255	0.067	4.391	0.759
56	0.429	0.009	64.985	0.417	0.197	0.014	3.488	0.556
64	0.437	0.007	76.540	0.341	0.130	0.075	4.133	0.112
72	0.440	0.008	72.870	0.321	0.130	0.061	4.180	0.502

Variant 5 (0.456 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.098	0.000	105.700	1.980	6.391	0.000	5.811	0.316
8	0.130	0.001	102.700	0.990	5.579	0.554	5.796	0.796
16	0.159	0.004	98.280	0.099	2.991	2.591	5.530	0.421
24	0.201	0.006	105.700	1.838	2.949	0.430	4.364	1.026
32	0.269	0.009	104.650	0.212	1.002	0.279	4.590	0.359
40	0.363	0.017	97.315	0.177	0.060	0.008	4.191	0.278
48	0.450	0.017	85.280	0.311	0.278	0.251	4.223	0.200
56	0.456	0.017	71.000	0.240	0.379	0.144	3.375	0.457
64	0.462	0.016	74.910	0.302	0.263	0.142	3.790	0.271
72	0.466	0.019	70.090	0.400	0.240	0.113	3.450	0.255

Variant 6 (0.913 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.098	0.000	107.450	0.071	12.688	0.088	5.897	0.293
8	0.128	0.002	103.600	1.697	11.540	0.268	5.427	0.273
16	0.168	0.003	108.550	1.344	9.989	0.459	4.799	0.462
24	0.206	0.007	110.750	0.071	8.334	0.790	4.404	0.475
32	0.280	0.018	102.700	1.556	5.496	1.188	4.583	0.087
40	0.384	0.035	70.350	0.453	2.916	0.863	4.198	0.257
48	0.496	0.022	59.000	0.636	1.274	0.840	3.529	0.770
56	0.505	0.023	38.885	0.078	0.837	0.579	2.749	0.872
64	0.512	0.024	34.120	0.056	1.140	0.104	3.467	0.495
72	0.520	0.026	25.910	0.040	0.903	0.105	3.237	0.475

Variant 7 (1.141 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.096	0.000	107.150	0.495	16.104	0.220	5.724	0.181
8	0.132	0.004	108.550	2.051	14.609	0.900	5.429	0.915
16	0.158	0.005	109.550	0.354	13.362	0.850	5.122	0.511
24	0.199	0.004	100.450	0.212	11.870	0.746	4.818	0.694
32	0.265	0.011	93.055	0.304	9.979	0.854	5.001	0.389
40	0.363	0.010	73.155	0.276	9.817	3.156	4.106	1.006
48	0.459	0.017	73.080	0.156	5.907	1.120	3.980	0.304
56	0.467	0.018	29.835	0.163	4.298	0.693	2.790	1.253
64	0.476	0.016	59.810	0.102	2.500	0.608	3.553	0.396
72	0.484	0.012	50.950	0.111	1.663	0.582	3.270	0.445

SD-standard deviation

Nitrogen enrichment experiment: Data for *Scenedesmus subspicatus*

Variant 8 (2.282 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.100	0.000	107.050	0.354	31.963	0.016	5.746	0.419
8	0.130	0.004	106.800	0.000	29.657	1.767	5.661	0.454
16	0.161	0.007	106.500	0.424	32.195	5.587	5.520	0.157
24	0.212	0.006	92.140	0.113	31.790	5.723	4.965	0.430
32	0.279	0.002	111.150	0.212	28.146	3.326	4.979	0.527
40	0.379	0.010	91.150	0.028	24.791	2.484	4.895	0.213
48	0.479	0.016	57.450	0.467	22.147	2.697	4.394	0.684
56	0.494	0.015	86.870	0.071	18.373	0.813	3.045	1.512
64	0.504	0.015	79.023	0.057	16.970	0.052	3.950	0.440
72	0.515	0.014	77.032	0.044	15.663	0.503	3.610	0.477

Variant 9 (3.424 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.097	0.000	107.350	0.071	47.720	0.365	5.936	0.194
8	0.132	0.009	100.700	0.707	45.071	1.723	5.426	1.314
16	0.164	0.003	107.900	0.849	42.640	1.897	5.238	0.881
24	0.214	0.004	93.605	0.035	38.592	1.256	5.455	1.168
32	0.287	0.008	113.050	0.778	36.248	2.780	4.918	0.271
40	0.387	0.012	113.800	1.131	32.516	2.261	3.737	0.658
48	0.481	0.008	74.305	0.445	32.885	5.823	4.200	1.106
56	0.491	0.008	67.170	0.382	29.538	5.773	3.964	1.188
64	0.505	0.008	63.700	0.090	26.293	4.015	4.100	0.345
72	0.517	0.007	60.323	0.077	23.967	3.902	3.550	0.435

Variant 10 (4.565 µmolN l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.099	0.000	107.050	1.061	64.600	1.199	5.730	0.482
8	0.129	0.006	108.700	0.849	63.816	1.274	6.143	1.125
16	0.160	0.003	105.850	3.041	62.603	1.455	6.255	2.204
24	0.202	0.005	105.900	0.141	62.575	3.039	5.334	0.640
32	0.266	0.016	104.550	0.636	61.141	1.886	2.962	2.643
40	0.352	0.037	82.125	0.544	58.529	2.936	5.925	3.346
48	0.432	0.033	81.625	0.304	56.742	3.110	4.031	0.389
56	0.446	0.032	31.380	0.396	52.765	4.137	3.533	0.502
64	0.458	0.031	58.990	0.390	48.900	5.841	3.747	0.636
72	0.479	0.026	48.054	0.340	43.160	2.702	3.803	0.374

SD-standard deviation

Phosphorus enrichment experiment: Data for *Chlorella vulgaris*

Concentration of phosphorus source (PO_4^{2-}) in experimental variants for *Chlorella vulgaris*

variant	P (µmolL^{-1})	Exponential phase* (h)	
1	0.000	0	32
2	0.048	0	40
3	0.096	0	40
4	0.193	0	32
5	0.385	0	32
6	0.578	0	32

Variant 1 (0.000 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.089	0.005	195.833	2.303	15.902	0.248	-	-
8	0.119	0.004	185.900	0.707	15.053	0.098	-	-
16	0.168	0.008	190.133	1.537	14.370	0.562	-	-
24	0.211	0.004	187.200	7.354	13.387	0.988	-	-
32	0.255	0.003	181.367	17.360	12.114	0.376	-	-
40	0.277	0.002	180.600	10.050	11.341	0.557	-	-
48	0.300	0.007	177.930	8.800	10.434	0.699	-	-
56	0.315	0.003	174.500	8.300	9.777	0.032	-	-
64	0.327	0.005	167.010	1.600	9.476	0.235	-	-
72	0.345	0.010	165.200	10.220	9.148	0.273	-	-

Variant 2 (0.048 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.097	0.002	197.200	4.384	15.847	0.002	1.466	0.560
8	0.130	0.002	181.667	5.607	15.510	0.446	1.422	0.600
16	0.184	0.010	185.500	1.697	13.648	1.044	1.450	0.450
24	0.269	0.011	192.467	5.412	12.664	1.261	2.462	0.670
32	0.394	0.018	182.167	14.288	12.584	2.056	1.789	0.680
40	0.529	0.010	182.350	3.041	9.580	3.939	1.079	0.900
48	0.634	0.023	179.000	1.344	7.660	3.756	0.914	0.490
56	0.678	0.063	176.030	11.000	6.370	4.307	1.482	0.650
64	0.750	0.026	171.650	5.800	6.926	5.516	1.541	0.320
72	0.771	0.054	168.540	9.100	5.429	4.906	0.738	0.600

Variant 3 (0.096 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.087	0.002	216.500	5.889	15.890	0.113	2.985	0.570
8	0.126	0.004	195.800	6.788	15.486	0.508	2.835	0.700
16	0.169	0.005	185.750	1.626	14.557	0.449	2.740	0.450
24	0.258	0.012	170.900	3.818	13.405	1.150	2.547	0.800
32	0.371	0.020	162.550	4.900	11.254	1.783	2.547	0.320
40	0.510	0.031	170.100	10.010	9.818	2.498	2.525	0.650
48	0.613	0.028	161.500	11.000	6.984	2.739	2.251	0.540
56	0.657	0.046	158.700	6.900	6.171	3.981	2.481	0.800
64	0.732	0.043	154.870	8.000	5.956	4.874	2.835	0.430
72	0.765	0.024	149.850	6.900	4.155	4.628	2.740	0.200

SD-standard deviation

* the period of the exponential growth phase for algal cultures (the onset and the end)

Phosphorus enrichment experiment: Data for *Chlorella vulgaris*

Variant 4 (0.193 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.103	0.002	218.500	4.400	15.675	0.411	5.972	1.200
8	0.150	0.005	195.800	6.100	14.779	0.186	4.774	0.900
16	0.199	0.006	185.750	10.000	14.216	0.109	5.064	0.980
24	0.301	0.007	170.900	10.900	12.633	0.900	5.262	0.780
32	0.426	0.024	159.550	8.700	10.448	1.592	4.121	0.690
40	0.548	0.005	155.100	6.900	9.265	3.342	5.521	3.010
48	0.642	0.007	151.480	5.500	6.871	3.903	3.259	0.340
56	0.702	0.012	147.900	3.900	5.790	4.694	2.478	0.700
64	0.734	0.007	144.220	11.000	3.421	2.463	1.883	0.980
72	0.765	0.013	145.000	12.100	0.988	0.197	2.496	0.500

Variant 5 (0.385 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.109	0.008	209.467	8.751	15.914	0.027	11.888	1.200
8	0.149	0.004	195.950	14.071	15.086	0.234	11.543	0.900
16	0.203	0.002	184.300	1.838	13.992	0.132	11.929	2.980
24	0.305	0.008	161.110	12.572	12.623	0.930	11.625	1.780
32	0.431	0.013	149.100	11.879	10.398	1.824	9.633	0.690
40	0.528	0.003	133.200	3.069	8.247	3.006	10.530	3.010
48	0.638	0.002	130.090	5.000	6.661	3.419	9.421	0.340
56	0.696	0.017	126.550	9.100	6.049	4.971	9.292	0.700
64	0.766	0.018	124.060	7.500	5.160	5.047	9.027	0.980
72	0.775	0.009	121.110	6.900	4.557	4.784	8.338	0.500

Variant 6 (0.578 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.100	0.005	219.467	5.889	15.923	0.063	23.724	1.570
8	0.146	0.012	185.950	6.079	15.567	0.754	22.893	2.700
16	0.195	0.015	174.300	1.163	14.085	0.209	23.228	2.450
24	0.295	0.025	166.110	3.282	11.910	3.243	23.228	1.800
32	0.425	0.021	155.100	4.090	11.115	2.580	19.813	2.320
40	0.538	0.029	153.430	10.100	10.216	1.202	23.064	3.650
48	0.624	0.008	150.330	10.910	7.683	4.458	19.657	0.540
56	0.685	0.028	147.760	6.900	5.948	4.740	20.925	0.180
64	0.741	0.020	144.210	8.900	5.591	5.811	19.652	0.430
72	0.780	0.019	140.001	6.900	5.275	5.737	19.274	0.620

SD-standard deviation

Phosphorus enrichment experiment: Data for *Scenedesmus subspicatus*

Concentration of phosphorus source (PO_4^{2-}) in experimental variants for *Scenedesmus subspicatus*

variant	P (µmolL^{-1})	Exponential phase* (h)	
1	0.000	0	32
2	0.048	0	32
3	0.096	0	32
4	0.193	0	32
5	0.385	0	32
6	0.578	0	40

Variant 1 (0.000 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.098	0.000	221.833	29.780	15.903	1.800	-	-
8	0.149	0.000	195.900	18.770	15.029	1.400	-	-
16	0.216	0.006	190.133	16.010	12.819	0.510	-	-
24	0.297	0.019	187.200	18.730	9.142	1.590	-	-
32	0.401	0.038	175.367	25.860	8.953	0.930	-	-
40	0.537	0.019	173.000	14.110	8.715	0.541	-	-
48	0.632	0.009	170.900	15.790	6.268	0.300	-	-
56	0.681	0.013	167.500	19.540	3.203	0.320	-	-
64	0.740	0.031	163.900	20.000	0.129	0.040	-	-
72	0.751	0.027	161.050	12.300	0.100	0.010	-	-

Variant 2 (0.048 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.096	0.000	221.200	32.500	16.035	0.160	3.703	1.030
8	0.147	0.001	181.667	25.110	14.599	0.200	1.591	0.390
16	0.208	0.006	185.500	18.600	10.907	0.500	1.661	0.610
24	0.295	0.011	192.467	24.500	7.575	0.260	1.465	0.210
32	0.425	0.016	181.167	9.910	3.928	0.050	1.520	0.140
40	0.560	0.027	182.350	19.770	2.966	0.040	1.512	0.090
48	0.646	0.096	177.220	12.210	1.959	0.300	1.388	0.030
56	0.654	0.119	174.560	4.400	1.299	0.010	1.107	0.120
64	0.629	0.123	170.320	5.700	0.904	0.040	1.196	0.040
72	0.640	0.124	166.900	6.700	0.240	0.020	1.450	0.020

Variant 3 (0.096 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.099	0.000	219.500	23.200	16.011	0.170	3.988	1.050
8	0.150	0.001	199.800	15.110	15.125	0.460	2.934	0.439
16	0.208	0.002	195.750	18.600	11.563	0.150	2.560	0.670
24	0.306	0.014	179.900	9.500	8.954	0.360	2.015	0.240
32	0.401	0.009	162.550	15.100	2.952	0.140	0.010	0.014
40	0.448	0.024	160.100	16.770	4.359	0.049	0.086	0.019
48	0.460	0.025	161.320	12.210	1.959	0.300	1.086	0.030
56	0.472	0.017	158.800	14.400	1.299	0.010	1.003	0.120
64	0.514	0.021	154.780	8.700	0.904	0.040	0.586	0.040
72	0.534	0.020	155.050	3.200	0.750	0.040	0.400	0.020

SD-standard deviation

* the period of the exponential growth phase for algal cultures (the onset and the end)

Phosphorus enrichment experiment: Data for *Scenedesmus subspicatus*

Variant 4 (0.193 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.096	0.000	219.467	20.200	15.975	2.100	7.018	0.250
8	0.149	0.003	185.950	12.110	13.083	2.400	5.987	0.090
16	0.207	0.008	174.300	12.600	9.089	1.100	5.119	0.060
24	0.301	0.031	171.110	7.590	6.596	1.300	4.030	0.210
32	0.438	0.010	160.100	9.910	3.960	0.400	1.602	0.120
40	0.508	0.055	163.090	12.770	1.020	0.049	1.729	0.019
48	0.550	0.135	158.650	12.210	1.021	0.003	2.172	0.030
56	0.568	0.155	155.410	14.100	0.186	0.010	2.006	0.100
64	0.633	0.146	154.300	8.170	0.309	0.020	1.173	0.040
72	0.650	0.150	152.000	10.000	0.400	0.001	1.090	0.100

Variant 5 (0.385 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.097	0.000	221.833	23.200	15.968	1.120	12.591	0.540
8	0.149	0.001	185.900	25.110	14.829	1.470	11.286	0.930
16	0.222	0.001	190.133	28.600	11.763	1.120	9.940	0.670
24	0.281	0.005	177.200	14.050	8.574	0.900	9.614	0.520
32	0.439	0.006	161.367	18.100	2.024	0.240	8.087	0.140
40	0.590	0.011	158.600	16.000	0.322	0.040	5.816	0.019
48	0.577	0.016	154.900	12.210	0.185	0.003	6.755	0.030
56	0.596	0.029	149.990	14.100	0.272	0.010	5.091	0.120
64	0.593	0.064	147.860	8.170	0.370	0.040	5.922	0.500
72	0.606	0.067	146.780	9.000	0.510	0.010	3.980	0.430

Variant 6 (0.578 mmolP l^{-1})

Time (h)	OD_{678} ±SD		DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.097	0.000	209.200	23.920	15.950	1.010	18.699	1.540
8	0.146	0.001	181.667	17.110	15.407	1.040	18.271	1.930
16	0.215	0.011	185.500	23.600	11.958	0.810	17.918	0.790
24	0.301	0.002	192.467	21.500	8.566	0.450	17.658	0.920
32	0.404	0.001	172.167	20.510	5.392	0.240	16.890	0.940
40	0.582	0.019	160.350	19.770	1.854	0.030	13.850	0.190
48	0.594	0.029	162.090	2.210	0.261	0.010	15.755	0.030
56	0.620	0.041	158.600	14.100	0.611	0.020	14.976	0.120
64	0.677	0.087	153.600	8.170	0.192	0.040	12.200	0.500
72	0.690	0.091	151.670	6.100	0.320	0.090	11.800	0.870

SD-standard deviation

Light-Temperature experiment with *Chlorella vulgaris*

Experimental conditions for *Chlorella vulgaris*

Variant	T^0C	Light($\mu molm^{-2}s^{-1}$)	Exponential phase* (h)	
1	5	78.3	40	80
2	5	62.7	8	88
3	5	47.0	32	72
4	5	31.3	16	64
5	5	15.7	16	72
6	5	7.8	24	80
7	10	78.3	24	72
8	10	62.7	8	72
9	10	47.0	24	80
10	10	31.3	24	96
11	10	15.7	0	80
12	10	7.8	8	80
13	15	78.3	0	56
14	15	62.7	0	56
15	15	47.0	8	56
16	15	31.3	0	56
17	15	15.7	24	88
18	15	7.8	40	88
19	20	78.3	24	56
20	20	62.7	16	56
21	20	47.0	32	64
22	20	31.3	24	64
23	20	15.7	8	80
24	20	7.8	8	72

Variant 1 (T:5^{0}C, L:78.3 $\mu molm^{-2}s^{-1}$)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.320	0.000	16.22	0.10	5.607	0.011
8	0.323	0.002	16.07	0.06	5.598	0.012
16	0.325	0.003	15.86	0.13	5.081	0.054
24	0.329	0.001	15.78	0.18	4.854	0.307
32	0.335	0.002	15.61	0.30	4.951	0.675
40	0.348	0.003	15.18	0.49	5.197	0.504
48	0.373	0.002	14.67	0.39	4.351	0.364
56	0.396	0.002	14.34	0.19	4.951	0.097
64	0.426	0.007	13.94	0.29	4.855	0.996
72	0.458	0.004	13.61	0.83	4.911	1.270
80	0.488	0.005	12.29	0.78	4.891	0.631
88	0.507	0.003	10.82	0.24	4.911	0.238

Variant 2 (T:5^{0}C, L:62.7 $\mu molm^{-2}s^{-1}$)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.190	0.000	15.803	0.102	5.817	0.242
8	0.195	0.003	15.633	0.092	5.679	0.104
16	0.207	0.002	15.530	0.105	5.728	0.340
24	0.230	0.008	14.490	0.058	5.330	0.146
32	0.240	0.003	14.139	0.447	5.513	0.223
40	0.254	0.003	13.904	0.458	5.229	0.397
48	0.272	0.006	13.238	0.263	5.304	0.069
56	0.288	0.002	12.733	0.242	4.999	0.160
64	0.322	0.003	11.597	0.218	4.969	0.014
72	0.345	0.010	11.160	0.222	4.708	0.145
80	0.365	0.012	10.431	0.254	4.384	0.341
88	0.385	0.004	9.495	0.196	4.303	0.150

* the time (h) of the onset and the end of the exponential phase of growth.
SD-standard deviation

Light-Temperature experiment with *Chlorella vulgaris*

Variant 3 (T:5°C, L:47.0 μmolm^{-2}s^{-1})

Time (h)	OD$_{678}$ ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.256	0.000	16.22	0.17	6.14	0.14
8	0.258	0.000	15.78	0.53	5.55	0.90
16	0.259	0.002	14.90	0.67	4.97	0.24
24	0.262	0.001	14.37	0.44	5.49	0.28
32	0.271	0.005	13.17	0.32	4.88	1.03
40	0.279	0.004	12.31	0.30	4.80	0.65
48	0.293	0.005	11.36	0.31	4.24	0.44
56	0.311	0.006	10.85	0.25	5.00	0.81
64	0.326	0.007	10.34	0.16	3.83	0.53
72	0.340	0.006	9.38	0.18	3.10	0.08
80	0.348	0.007	6.88	0.58	3.00	0.10
88	0.367	0.005	4.88	0.63	2.97	0.13

Variant 4 (T:5°C, L:31.3 μmolm^{-2}s^{-1})

Time (h)	OD$_{678}$ ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.248	0.000	16.174	0.164	6.554	0.111
8	0.251	0.001	15.199	0.232	6.515	0.092
16	0.254	0.000	14.697	0.282	5.792	0.658
24	0.267	0.002	13.863	0.171	6.625	0.762
32	0.281	0.003	13.087	0.167	5.915	0.660
40	0.297	0.004	12.364	0.629	5.527	1.102
48	0.316	0.004	12.018	0.580	4.820	0.494
56	0.332	0.003	10.892	0.729	4.375	0.701
64	0.350	0.005	10.083	0.429	3.415	0.565
72	0.359	0.007	9.268	1.413	2.777	0.907
80	0.367	0.007	7.392	1.225	2.820	0.786
88	0.384	0.007	5.833	1.662	1.570	1.060

Variant 5 (T:5°C, L:15.7 μmolm^{-2}s^{-1})

Time (h)	OD$_{678}$ ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.306	0.000	16.039	0.166	5.844	0.597
8	0.307	0.001	15.848	0.236	5.217	1.511
16	0.310	0.002	15.384	0.337	4.627	0.728
24	0.325	0.001	14.679	0.544	4.296	0.695
32	0.334	0.001	13.914	0.204	3.155	1.249
40	0.346	0.002	13.301	0.139	2.999	0.705
48	0.357	0.008	12.828	0.266	2.786	1.755
56	0.368	0.007	12.242	0.591	3.300	0.547
64	0.376	0.011	11.517	0.649	2.653	0.968
72	0.392	0.010	10.280	1.057	2.536	1.459
80	0.401	0.009	8.267	0.912	2.300	0.555
88	0.407	0.010	6.530	1.308	1.903	0.705

Variant 6 (T:5°C, L:7.8 μmolm^{-2}s^{-1})

Time (h)	OD$_{678}$ ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.200	0.000	16.185	0.521	5.932	0.230
8	0.201	0.001	15.629	0.230	5.822	0.157
16	0.202	0.001	15.474	0.143	5.855	0.151
24	0.204	0.002	15.322	0.131	5.653	0.428
32	0.207	0.002	15.035	0.115	5.646	0.357
40	0.210	0.002	14.784	0.167	5.423	0.213
48	0.215	0.002	14.460	0.116	5.336	0.302
56	0.219	0.003	14.154	0.295	5.614	0.214
64	0.223	0.003	13.508	0.152	5.275	0.101
72	0.227	0.003	13.191	0.224	5.101	0.093
80	0.230	0.002	12.471	0.219	4.939	0.167
88	0.232	0.003	11.843	0.214	4.656	0.172

Light-Temperature experiment with *Chlorella vulgaris*

Variant 7 (T:10°C, L:78.3 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.159	0.000	15.437	0.152	5.60	0.04
8	0.172	0.004	15.486	0.143	5.56	0.18
16	0.189	0.001	15.376	0.282	5.55	0.15
24	0.211	0.001	14.132	0.414	5.00	0.55
32	0.245	0.006	12.550	0.629	-	-
40	0.292	0.004	11.430	0.536	3.79	0.07
48	0.344	0.009	9.297	0.675	3.75	0.17
56	0.447	0.013	6.592	0.524	3.37	0.12
64	0.572	0.023	2.266	0.475	2.26	0.04
72	0.685	0.026	0.229	0.141	1.80	0.09
80	0.721	0.019	1.266	0.475	0.80	0.10
88	0.737	0.025	0.758	0.302	0.30	0.16

Variant 8 (T:10°C, L:62.7 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.156	0.000	15.964	0.034	5.603	0.337
8	0.165	0.001	15.812	0.001	5.470	0.377
16	0.188	0.005	15.570	0.088	-	-
24	0.208	0.004	15.307	0.241	4.713	0.339
32	0.241	0.008	14.256	0.201	5.656	0.485
40	0.282	0.011	12.861	0.218	5.803	1.106
48	0.330	0.012	10.158	0.392	4.648	0.506
56	0.393	0.020	8.998	0.419	4.755	0.405
64	0.446	0.018	5.905	0.201	3.825	0.547
72	0.507	0.005	4.717	0.323	4.755	0.405
80	0.552	0.013	2.905	0.201	3.825	0.547
88	0.589	0.015	2.305	0.379	2.325	0.696

Variant 9 (T:10°C, L:47.0 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.170	0.000	15.894	0.118	6.355	0.798
8	0.179	0.001	15.410	0.201	5.940	0.826
16	0.196	0.004	15.054	0.263	5.700	0.429
24	0.210	0.003	14.319	0.155	6.624	1.011
32	0.233	0.004	13.370	0.417	6.159	0.512
40	0.264	0.002	12.105	0.204	5.355	1.127
48	0.299	0.004	10.306	1.096	4.405	1.122
56	0.360	0.010	9.022	1.050	3.549	-
64	0.418	0.024	7.175	0.719	4.973	0.421
72	0.492	0.039	5.827	1.045	4.615	1.567
80	0.551	0.039	5.208	0.801	4.199	0.445
88	0.608	0.046	3.578	1.968	2.550	0.396

Variant 10 (T:10°C, L:31.3 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.163	0.000	15.981	0.137	5.032	1.207
8	0.172	0.004	15.582	0.152	5.602	1.320
16	0.183	0.003	14.969	0.344	5.052	0.271
24	0.190	0.000	13.963	0.314	-	-
32	0.205	0.002	13.150	0.499	5.593	0.699
40	0.228	0.004	12.019	0.480	5.140	0.491
48	0.255	0.006	10.751	0.878	-	-
56	0.280	0.007	9.328	0.710	3.714	1.375
64	0.313	0.011	8.158	0.302	4.787	1.220
72	0.351	0.018	7.275	0.616	3.766	1.129
80	0.411	0.025	6.337	0.598	3.994	1.862
88	0.469	0.031	5.148	0.669	3.473	0.384
96	0.513	0.033	4.493	0.718	3.711	0.728

Light-Temperature experiment with *Chlorella vulgaris*

Variant 11(T:10°C, L:15.7 µmolm⁻²s⁻¹)

Time (h)	OD678 ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.160	0.000	15.875	0.100	6.076	0.397
8	0.174	0.001	15.544	0.312	5.727	0.585
16	0.191	0.002	15.496	0.240	5.648	0.439
24	0.206	0.003	14.799	0.213	5.728	0.550
32	0.226	0.003	14.200	0.241	-	-
40	0.253	0.003	13.906	0.115	5.068	0.672
48	0.282	0.003	13.753	0.116	5.324	0.095
56	0.313	0.005	13.248	0.187	5.169	0.272
64	0.344	0.003	12.799	0.276	4.831	0.363
72	0.378	0.008	10.799	0.558	4.746	0.429
80	0.407	0.016	9.828	0.449	4.831	0.363
88	0.424	0.013	8.348	1.147	4.746	0.429

Variant 12(T:10°C, L:7.8 µmolm⁻²s⁻¹)

Time (h)	OD678 ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.274	0.000	16.206	0.110	6.241	0.251
8	0.282	0.002	16.148	0.116	6.652	0.102
16	0.287	0.003	16.065	0.104	6.227	0.235
24	0.292	0.003	15.939	0.100	6.651	0.073
32	0.299	0.001	15.821	0.106	6.038	0.068
40	0.301	0.002	15.611	0.172	6.339	0.294
48	0.311	0.003	15.436	0.154	6.370	0.268
56	0.318	0.003	15.100	0.171	6.380	0.137
64	0.325	0.003	14.798	0.404	6.345	0.174
72	0.332	0.002	14.440	0.395	6.241	0.235
80	0.339	0.004	14.273	0.463	6.320	0.385
88	0.349	0.005	13.821	0.529	6.518	0.061

Variant 13(T:15°C, L:78.3 µmolm⁻²s⁻¹)

Time (h)	OD678 ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.099	0.000	16.376	0.231	5.09	0.185
8	0.130	0.002	15.440	0.521	5.09	0.315
16	0.166	0.006	13.225	0.217	4.55	0.355
24	0.205	0.002	10.945	0.054	4.06	0.470
32	0.269	0.009	8.560	0.125	4.32	0.371
40	0.363	0.007	5.483	0.343	4.04	0.827
48	0.549	0.023	2.932	0.114	3.24	0.461
56	0.667	0.018	0.942	0.229	2.66	0.210
64	0.707	0.016	0.118	0.037	2.01	0.129
72	0.741	0.015	0.018	0.005	1.34	0.112
80	0.785	0.017	0.143	0.044	1.06	0.136

Variant 14(T:15°C, L:62.7 µmolm⁻²s⁻¹)

Time (h)	OD678 ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.100	0.000	16.358	0.047	5.727	0.366
8	0.139	0.003	15.638	0.087	5.348	0.284
16	0.177	0.005	14.264	0.253	5.323	0.161
24	0.220	0.010	11.733	0.154	5.375	0.595
32	0.307	0.007	9.934	0.632	4.662	0.091
40	0.428	0.010	6.950	0.208	3.842	0.342
48	0.572	0.021	3.944	0.421	3.689	0.844
56	0.727	0.015	2.098	0.407	2.657	0.496
64	0.841	0.008	0.340	0.187	2.487	0.403
72	0.891	0.018	0.147	0.182	1.439	0.425
80	0.962	0.020	0.120	0.088	0.406	0.231

Light-Temperature experiment with *Chlorella vulgaris*

Variant 15(T:15°C, L:47.0 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.097	0.000	16.451	0.147	6.130	0.305
8	0.118	0.004	15.661	0.068	5.883	0.532
16	0.148	0.005	14.372	0.242	5.509	0.056
24	0.185	0.007	12.871	0.346	6.568	0.894
32	0.251	0.012	11.799	0.788	4.473	1.139
40	0.350	0.001	9.473	0.502	4.671	1.268
48	0.495	0.014	7.303	0.968	5.987	1.756
56	0.653	0.019	3.184	0.458	4.402	0.550
64	0.778	0.032	0.864	0.624	4.223	0.670
72	0.880	0.036	0.868	1.222	3.328	0.394
80	0.932	0.031	0.512	0.326	1.743	0.508

Variant 16(T:15°C, L:31.3 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.092	0.000	16.175	0.105	5.178	0.760
8	0.132	0.003	15.744	0.383	3.993	0.226
16	0.152	0.002	14.300	0.658	4.640	0.860
24	0.177	0.006	13.190	0.952	4.368	1.461
32	0.249	0.004	12.136	0.586	4.541	1.131
40	0.326	0.006	10.777	0.685	4.588	1.651
48	0.424	0.008	8.563	0.189	3.033	0.243
56	0.530	0.008	6.818	0.286	3.189	0.488
64	0.616	0.004	3.672	0.168	2.963	0.398
72	0.661	0.015	2.590	0.256	2.575	0.285
80	0.701	0.005	2.078	0.068	2.575	0.285

Variant 17(T:15°C, L:15.7 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.103	0.000	15.786	0.170	6.199	0.737
8	0.114	0.001	15.641	0.176	5.709	0.230
16	0.128	0.002	15.507	0.292	5.355	0.293
24	0.139	0.001	14.674	0.423	5.984	0.514
32	0.167	0.005	14.526	0.244	6.498	0.466
40	0.188	0.004	14.199	0.146	-	-
48	0.223	0.003	13.654	0.452	5.419	0.692
56	0.268	0.005	12.612	0.154	5.844	0.771
64	0.313	0.005	11.465	0.295	5.704	0.085
72	0.431	0.013	10.038	0.316	5.424	0.423
80	0.491	0.016	8.607	0.391	-	-
88	0.602	0.020	7.518	0.361	5.176	0.599

Variant 18(T:15°C, L:7.8 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.113	0.000	-	-	5.65	0.31
8	0.118	0.001	-	-	5.70	0.35
16	0.123	0.001	-	-	5.73	0.42
24	0.135	0.007	15.544	0.312	6.29	0.56
32	0.141	0.009	15.496	0.240	5.19	0.52
40	0.143	0.007	14.799	0.213	5.21	0.33
48	0.151	0.009	14.200	0.241	5.00	0.30
56	0.163	0.007	13.921	0.099	4.80	0.53
64	0.181	0.008	13.753	0.116	4.76	0.80
72	0.205	0.010	13.248	0.187	4.81	0.84
80	0.227	0.012	12.799	0.276	4.84	0.21
88	0.251	0.020	12.328	0.163	3.24	0.82

Light-Temperature experiment with *Chlorella vulgaris*

Variant 19(T:20°C, L:78.3 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.080	0.000	16.054	0.254	5.351	0.597
8	0.080	0.004	15.993	0.183	5.105	0.537
16	0.090	0.007	15.521	0.162	5.085	0.318
24	0.109	0.006	14.892	0.187	4.955	0.302
32	0.155	0.011	13.665	0.356	4.813	0.402
40	0.200	0.013	11.836	0.323	4.736	0.521
48	0.292	0.025	9.661	0.382	4.551	0.180
56	0.394	0.030	9.165	0.358	4.103	0.062
64	0.474	0.034	8.336	0.271	3.235	0.140
72	0.558	0.046	7.661	0.382	2.536	0.157
80	0.619	0.060	7.093	0.017	1.314	0.421

Variant 20(T:20°C, L:62.7 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.099	0.000	14.86	0.10	5.819	0.135
8	0.112	0.001	14.68	0.03	5.534	0.158
16	0.131	0.002	16.32	0.08	5.584	0.277
24	0.174	0.014	14.69	0.32	5.773	0.155
32	0.223	0.016	13.26	0.44	5.861	0.152
40	0.313	0.029	11.11	0.61	5.585	0.093
48	0.417	0.040	6.56	0.57	5.389	0.104
56	0.534	0.045	3.32	0.46	5.132	0.262
64	0.643	0.052	1.84	0.69	4.628	0.256
72	0.752	0.057	1.11	0.13	3.882	0.681
80	0.796	0.046	0.64	0.22	3.128	0.633

Variant 21(T:20°C, L:47.0 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.107	0.000	16.078	0.052	5.568	0.090
8	0.116	0.005	15.767	0.105	5.392	0.093
16	0.126	0.009	15.068	0.145	5.502	0.122
24	0.135	0.006	15.024	0.452	5.555	0.189
32	0.154	0.006	13.686	0.287	5.196	0.096
40	0.207	0.010	12.419	0.131	5.013	0.041
48	0.291	0.016	10.794	0.193	4.936	0.170
56	0.387	0.024	8.495	0.244	4.448	0.130
64	0.513	0.032	5.921	0.148	3.958	0.067
72	0.631	0.028	3.852	0.470	3.440	0.252
80	0.709	0.023	2.185	0.204	2.555	0.333

Variant 22(T:20°C, L:31.3 μmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.104	0.000	15.990	0.065	5.693	0.043
8	0.114	0.003	16.020	0.177	5.554	0.069
16	0.121	0.005	15.451	0.109	5.637	0.195
24	0.132	0.004	15.041	0.596	5.693	0.334
32	0.167	0.008	14.066	0.129	5.508	0.314
40	0.218	0.002	12.745	0.090	5.325	0.227
48	0.279	0.009	11.360	0.222	4.981	0.244
56	0.348	0.007	9.481	0.411	4.934	0.109
64	0.437	0.015	7.490	0.278	4.340	0.106
72	0.527	0.017	5.647	0.434	4.064	0.097
80	0.589	0.009	4.894	0.697	3.648	0.403

Light-Temperature experiment with *Chlorella vulgaris*

Variant 23(T:20^{0}C, L:15.7 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.107	0.000	15.786	0.170	5.881	0.093
8	0.119	0.002	15.507	0.292	5.879	0.092
16	0.142	0.002	14.754	0.341	5.006	0.632
24	0.168	0.005	14.199	0.146	4.932	0.468
32	0.189	0.003	13.654	0.452	5.202	0.572
40	0.236	0.015	12.612	0.154	4.765	0.494
48	0.261	0.005	11.465	0.295	4.893	0.492
56	0.312	0.004	10.038	0.316	5.059	0.404
64	0.373	0.004	8.607	0.391	5.172	0.387
72	0.438	0.008	7.518	0.361	4.669	0.585
80	0.516	0.009	6.300	0.346	5.189	0.484
88	0.592	0.018	5.003	0.124	5.265	0.595

Variant 24(T:20^{0}C, L:7.8 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.130	0.000	15.853	0.045	4.880	0.471
8	0.115	0.002	15.577	0.206	4.589	0.482
16	0.121	0.002	15.242	0.326	4.556	0.432
24	0.128	0.003	15.114	0.389	3.541	2.413
32	0.132	0.003	14.730	0.081	4.489	0.557
40	0.139	0.007	14.331	0.323	4.495	0.437
48	0.142	0.006	14.211	0.314	5.059	0.693
56	0.150	0.009	14.141	0.377	0.000	0.000
64	0.157	0.009	13.717	0.255	5.014	0.653
72	0.163	0.006	13.501	0.107	4.313	0.540
80	0.165	0.005	13.462	1.304	5.029	0.723

Light-Temperature experiment with *Chlorella vulgaris*

***Chlorella vulgaris*: Oxygen production (mg l^{-1}) ±SD**

Time (hrs)	variant 1		variant 2		variant 3		variant 4	
2	0.0763	0.002	0.0619	0.002	0.0488	0.007	0.0493	0.004
4	0.1420	0.009	0.1251	0.002	0.0935	0.010	0.0870	0.013
6	0.1910	0.002	0.1879	0.005	0.1382	0.005	0.1354	0.016
8	0.2622	0.003	0.2498	0.005	0.1807	0.003	0.1706	0.019

Time (hrs)	variant 5		variant 6		variant 7		variant 8	
2	0.0240	0.001	0.0153	0.002	0.1381	0.025	0.1047	0.009
4	0.0496	0.003	0.0292	0.002	0.2227	0.015	0.2033	0.023
6	0.0756	0.004	0.0429	0.005	0.3182	0.009	0.2952	0.000
8	0.1088	0.002	0.0686	0.005	0.3912	0.065	0.3681	0.052

Time (hrs)	variant 9		variant 10		variant 11		variant 12	
2	0.0810	0.007	0.0664	0.005	0.0628	0.010	0.0271	0.006
4	0.1539	0.016	0.1686	0.013	0.1016	0.029	0.0554	0.006
6	0.2644	0.056	0.2255	0.009	0.1743	0.026	0.0841	0.016
8	0.3544	0.017	0.2534	0.008	0.2391	0.025	0.0961	0.017

Time (hrs)	variant 13		variant 14		variant 15		variant 16	
1	0.128	0.009	0.148	0.042	0.123	0.015	0.116	0.025
2	0.211	0.020	0.210	0.023	0.203	0.022	0.187	0.017
3	0.297	0.028	0.315	0.029	0.292	0.013	0.269	0.023
4	0.350	0.038	0.410	0.025	0.391	0.008	0.347	0.011
5	0.421	0.024	0.493	0.050	0.473	0.009	0.389	0.029
6	0.500	0.015	0.550	0.092	0.535	0.017	0.492	0.030
7	0.590	0.044	0.701	0.027	0.645	0.055	0.560	0.027
8	0.651	0.038	0.766	0.113	0.708	0.025	0.630	0.015

Time (hrs)	variant 17		variant 18		variant 19		variant 20	
1	0.061	0.016	0.029	0.003	0.1224	0.024	0.0966	0.003
2	0.074	0.007	0.041	0.002	0.2139	0.012	0.1812	0.025
3	0.106	0.008	0.062	0.006	0.3137	0.022	0.2511	0.010
4	0.116	0.007	0.075	0.005	0.4134	0.024	0.3117	0.034
5	0.144	0.030	0.089	0.001	0.4936	0.045	0.4233	0.034
6	0.168	0.027	0.122	0.010	0.5625	0.039	0.4972	0.021
7		0.000	0.127	0.010	0.6652	0.016	0.6062	0.069
8	0.196	0.048	0.145	0.008	0.7391	0.012	0.6667	0.068

Time (hrs)	variant 21		variant 22		variant 23		variant 24	
1	0.1175	0.008	0.1341	0.020	0.0700	0.006	0.0196	0.004
2	0.1737	0.020	0.1487	0.005	0.1079	0.008	0.0377	0.003
3	0.2258	0.011	0.1551	0.019	0.1281	0.008	0.0580	0.004
4	0.2978	0.038	0.2838	0.023	0.1942	0.035	0.0704	0.006
5	0.4560	0.019	0.3445	0.051	0.1894	0.028	0.0851	0.004
6	0.4613	0.071	0.3885	0.102	0.2563	0.053	0.0973	0.007
7	0.5012	0.039	0.4166	0.028	0.2260	0.033	0.1164	0.008
8	0.5512	0.027	0.5390	0.116	0.3553	0.008	0.1221	0.010

Light-Temperature experiment with *Scenedesmus subspicatus*

Experimental conditions for *Scenedesmus subspicatus*

Variant	T^0C	Light(µmolm^{-2}s^{-1})	Exponential phase* (h)	
1	5	78.3	8	64
2	5	62.7	8	72
3	5	47.0	24	72
4	5	31.3	0	24
5	5	15.7	24	56
6	5	7.8	16	80
7	10	78.3	0	40
8	10	62.7	24	72
9	10	47.0	16	88
10	10	31.3	8	48
11	10	15.7	24	88
12	10	7.8	0	80
13	15	78.3	24	80
14	15	62.7	0	64
15	15	47.0	0	56
16	15	31.3	0	80
17	15	15.7	16	88
18	15	7.8	16	96
19	20	78.3	0	40
20	20	62.7	0	40
21	20	47.0	0	40
22	20	31.3	0	56
23	20	15.7	0	80
24	20	7.8	40	104

Variant 1 (T:5^{0}C, L:78.3 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.166	0.000	15.964	0.039	5.97	0.141
8	0.165	0.007	15.835	0.065	5.95	0.431
16	0.167	0.011	15.672	0.091	6.08	0.093
24	0.172	0.013	15.405	0.133	6.21	0.152
32	0.177	0.011	15.283	0.214	5.99	0.221
40	0.184	0.010	15.118	0.145	6.04	0.221
48	0.192	0.009	15.015	0.202	6.06	0.186
56	0.200	0.010	14.803	0.170	5.85	0.411
64	0.205	0.007	14.531	0.124	6.07	0.103
72	0.195	0.007	14.350	0.131	6.07	0.111
80	0.198	0.001	14.134	0.189	5.84	0.170
88	0.200	0.005	13.857	0.296	5.89	0.067

Variant 2 (T:5^{0}C, L:62.7 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.207	0.000	15.833	0.154	5.956	0.015
8	0.207	0.007	15.840	0.113	5.928	0.035
16	0.221	0.003	15.522	0.187	5.665	0.303
24	0.235	0.003	15.293	0.142	5.916	0.257
32	0.240	0.001	15.158	0.097	5.812	0.238
40	0.252	0.003	14.667	0.167	5.935	0.150
48	0.266	0.004	14.076	0.088	5.856	0.148
56	0.273	0.004	13.951	0.088	5.634	0.145
64	0.280	0.002	13.637	0.106	5.656	0.219
72	0.290	0.003	13.316	0.065	5.459	0.206
80	0.285	0.003	13.387	0.173	5.498	0.183
88	0.293	0.007	12.316	0.151	5.377	0.256

Light-Temperature experiment with *Scenedesmus subspicatus*

Variant 3 (T:5°C, L:47.0 µmolm⁻²s⁻¹)

Time (h)	OD_{678}	±SD	N (mg l⁻¹)	±SD	P (mg l⁻¹)	±SD
0	0.202	0.000	16.166	0.150	5.782	0.815
8	0.202	0.001	16.092	0.141	5.564	0.816
16	0.203	0.002	15.987	0.156	5.546	0.781
24	0.207	0.002	15.659	0.196	5.535	0.432
32	0.214	0.001	15.584	0.189	5.437	0.513
40	0.221	0.003	15.396	0.268	5.639	0.535
48	0.231	0.003	15.224	0.254	5.258	0.878
56	0.241	0.003	14.862	0.179	5.644	0.775
64	0.249	0.003	14.579	0.155	4.879	1.024
72	0.260	0.003	13.860	0.174	4.608	0.935
80	0.265	0.004	13.393	0.342	4.323	0.753
88	0.268	0.004	-	-	-	-

Variant 4 (T:5°C, L:31.3 µmolm⁻²s⁻¹)

Time (h)	OD_{678}	±SD	N (mg l⁻¹)	±SD	P (mg l⁻¹)	±SD
0	0.263	0.000	15.904	0.212	4.892	0.243
8	0.272	0.000	15.359	0.260	5.834	0.483
16	0.277	0.004	15.089	0.163	4.821	0.373
24	0.283	0.004	14.727	0.360	4.407	0.427
32	0.281	0.003	14.233	0.059	4.657	0.299
40	0.279	0.005	14.137	0.358	4.985	0.525
48	0.279	0.005	13.592	0.109	5.067	0.169
56	0.281	0.010	13.428	0.015	5.835	0.600
64	0.281	0.008	12.803	0.222	4.235	0.066
72	0.282	0.009	11.925	1.433	4.657	0.299
80	0.282	0.009	11.428	0.015	4.985	0.525
88	0.282	0.009	11.129	0.061	5.067	0.169

Variant 5 (T:5°C, L:15.7 µmolm⁻²s⁻¹)

Time (h)	OD_{678}	±SD	N (mg l⁻¹)	±SD	P (mg l⁻¹)	±SD
0	0.290	0.000	14.184	0.267	5.822	0.053
8	0.291	0.001	13.958	0.182	5.455	0.717
16	0.292	0.000	13.407	0.462	4.845	0.746
24	0.294	0.004	12.463	0.207	5.036	0.771
32	0.297	0.006	11.960	0.072	4.046	-
40	0.302	0.008	11.629	0.340	4.902	0.127
48	0.308	0.009	11.400	0.378	4.238	0.384
56	0.315	0.011	11.018	0.352	3.721	0.205
64	0.321	0.012	10.669	0.255	3.767	0.161
72	0.326	0.012	10.354	0.255	2.902	0.127
80	0.331	0.012	9.964	0.132	2.196	0.109
88	0.334	0.011	9.613	0.346	1.261	0.504

Variant 6 (T:5°C, L:7.8 µmolm⁻²s⁻¹)

Time (h)	OD_{678}	±SD	N (mg l⁻¹)	±SD	P (mg l⁻¹)	±SD
0	0.296	0.000	16.63	0.000	5.919	0.492
8	0.297	0.002	16.12	0.000	5.075	0.193
16	0.299	0.004	15.94	0.047	5.512	0.331
24	0.304	0.004	15.74	0.231	5.563	0.399
32	0.308	0.003	15.63	0.088	5.568	0.749
40	0.313	0.002	15.43	0.150	5.895	1.320
48	0.319	0.003	14.95	0.090	5.734	0.729
56	0.324	0.005	14.58	0.177	5.636	-
64	0.328	0.006	14.10	0.125	5.468	0.285
72	0.333	0.004	13.66	0.275	5.324	0.708
80	0.341	0.006	13.12	0.207	5.987	1.081
88	0.344	0.005	12.74	0.243	5.404	0.367

Light-Temperature experiment with *Scenedesmus subspicatus*

Variant 7 (T:10°C, L:78.3 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.161	0.000	15.826	0.096	5.564	0.140
8	0.188	0.006	15.084	0.510	5.855	0.382
16	0.214	0.002	13.567	0.357	5.436	0.151
24	0.241	0.003	11.693	0.260	4.934	0.159
32	0.272	0.003	10.277	0.369	5.287	0.095
40	0.311	0.001	8.737	0.248	5.262	0.298
48	0.328	0.002	6.452	0.221	5.120	0.066
56	0.361	0.007	5.674	0.278	4.904	0.067
64	0.388	0.005	4.376	0.379	5.046	0.144
72	0.414	0.009	2.480	0.462	5.004	0.110
80	0.426	0.012	2.865	0.787	4.707	0.079
88	0.439	0.015	2.200	0.484	4.800	0.152

Variant 8 (T:10°C, L:62.7 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.164	0.000	16.047	0.064	6.063	0.222
8	0.169	0.001	15.316	0.086	5.782	0.427
16	0.175	0.003	12.444	0.267	5.269	0.236
24	0.180	0.003	11.025	0.209	5.477	0.120
32	0.206	0.004	10.291	0.305	5.187	0.433
40	0.234	0.004	8.355	0.216	4.976	0.537
48	0.252	0.005	5.989	0.221	5.505	0.024
56	0.280	0.013	4.434	0.202	5.392	0.372
64	0.309	0.015	3.343	0.287	5.414	0.123
72	0.330	0.014	2.555	0.258	5.065	0.101
80	0.356	0.018	1.756	0.237	5.187	0.433
88	0.376	0.016	2.021	0.643	4.976	0.537

Variant 9 (T:10°C, L:47.0 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.166	0.000	15.868	0.105	6.113	0.325
8	0.168	0.001	15.819	0.232	5.947	0.244
16	0.171	0.003	14.559	0.210	5.520	0.653
24	0.176	0.002	14.327	0.282	4.970	0.440
32	0.183	0.001	13.633	0.188	4.940	0.396
40	0.187	0.002	13.345	0.269	4.551	0.436
48	0.194	0.003	12.747	0.243	4.874	0.270
56	0.203	0.002	11.962	0.388	4.632	0.491
64	0.209	0.001	11.650	0.248	4.557	0.620
72	0.219	0.002	10.552	0.221	4.376	0.318
80	0.226	0.004	9.176	0.626	4.580	0.535
88	0.238	0.004	7.764	0.667	4.608	0.410

Variant 10 (T:10°C, L:31.3 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.108	0.000	15.965	0.275	5.678	1.026
8	0.119	0.003	15.685	0.139	5.974	0.853
16	0.129	0.007	15.307	0.036	5.825	0.069
24	0.139	0.005	14.963	0.075	-	-
32	0.153	0.004	14.552	0.316	5.972	0.458
40	0.163	0.006	13.837	0.236	5.645	0.960
48	0.173	0.004	13.608	0.184	5.522	0.732
56	0.181	0.004	13.348	0.183	5.104	0.555
64	0.190	0.008	12.673	0.299	5.620	0.341
72	0.196	0.007	11.829	0.656	4.522	0.455
80	0.207	0.004	10.864	0.301	4.307	0.333
88	0.224	0.006	10.346	0.300	4.021	0.598

Light-Temperature experiment with *Scenedesmus subspicatus*

Variant 11(T:10°C, L:15.7 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.128	0.000	15.951	0.061	5.910	0.081
8	0.143	0.006	15.949	0.097	5.915	0.090
16	0.143	0.004	15.545	0.243	5.904	0.201
24	0.147	0.003	14.989	0.217	5.647	0.436
32	0.159	0.006	14.821	0.273	5.901	0.244
40	0.165	0.009	14.415	0.395	5.611	0.083
48	0.184	0.010	13.953	0.506	5.959	0.074
56	0.200	0.008	13.538	0.547	5.799	0.064
64	0.217	0.003	12.958	0.431	5.512	0.258
72	0.233	0.005	12.182	0.425	5.602	0.219
80	0.247	0.012	11.681	0.438	5.640	0.566
88	0.265	0.022	11.091	0.423	5.463	0.446

Variant 12(T:10°C, L:7.8 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.199	0.000	16.071	0.139	5.745	0.141
8	0.210	0.003	15.894	0.138	5.566	0.426
16	0.221	0.003	15.265	0.063	5.493	0.359
24	0.228	0.003	14.909	0.026	5.299	0.305
32	0.236	0.005	14.395	0.079	5.331	0.293
40	0.243	0.006	14.102	0.086	5.571	0.048
48	0.260	0.009	14.015	0.020	5.222	0.256
56	0.272	0.007	13.806	0.058	5.083	0.352
64	0.284	0.010	13.527	0.097	5.180	0.037
72	0.296	0.010	12.675	0.354	5.295	0.223
80	0.307	0.011	11.556	0.515	5.295	0.223
88	0.318	0.011	11.177	0.387	5.288	0.095

Variant 13(T:15°C, L:78.3 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.097	0.000	15.723	0.093	5.649	0.152
8	0.111	0.002	15.647	0.038	5.451	0.039
16	0.129	0.003	14.900	0.172	5.130	0.388
24	0.148	0.006	14.089	0.241	5.138	0.185
32	0.178	0.006	13.159	0.174	4.893	0.144
40	0.220	0.016	11.574	0.302	4.725	0.245
48	0.267	0.024	9.792	0.486	3.910	0.182
56	0.322	0.028	5.839	0.881	4.407	0.212
64	0.434	0.036	2.981	0.952	3.882	0.159
72	0.538	0.048	0.902	0.412	3.935	0.136
80	0.646	0.038	0.044	0.022	3.728	0.112
88	0.721	0.032	-	-	-	-

Variant 14(T:15°C, L:62.7 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.102	0.000	16.173	0.071	5.649	0.152
8	0.132	0.003	16.044	0.048	5.451	0.039
16	0.171	0.005	14.634	0.431	5.130	0.388
24	0.210	0.003	13.555	0.474	5.138	0.185
32	0.243	0.004	11.796	0.323	4.893	0.144
40	0.290	0.011	9.941	0.402	4.725	0.245
48	0.366	0.011	7.319	1.571	4.914	0.508
56	0.440	0.013	3.581	0.424	4.407	0.212
64	0.515	0.032	1.420	0.205	3.882	0.159
72	0.565	0.047	1.569	0.399	3.935	0.136
80	0.604	0.065	0.331	0.239	3.728	0.112
88	0.646	0.054	0.420	0.205	3.402	0.200

Light-Temperature experiment with *Scenedesmus subspicatus*

Variant 15(T:15°C, L:47.0 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.109	0.000	16.081	0.107	5.788	0.197
8	0.139	0.003	15.740	0.183	5.038	0.288
16	0.178	0.005	13.960	0.257	4.573	0.441
24	0.217	0.002	11.358	0.368	4.118	0.950
32	0.249	0.004	9.406	0.292	3.707	0.573
40	0.297	0.012	5.665	0.583	3.921	0.646
48	0.372	0.010	3.346	0.297	3.952	0.615
56	0.447	0.013	1.243	0.456	4.609	0.279
64	0.521	0.031	0.117	0.137	3.935	0.295
72	0.572	0.047	0.265	0.362	3.805	0.370
80	0.610	0.066	0.114	0.125	2.863	0.492
88	0.648	0.055	0.271	0.205	2.869	1.006

Variant 16(T:15°C, L:31.3 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.096	0.000	15.887	0.036	6.238	0.116
8	0.115	0.001	15.791	0.049	6.319	0.045
16	0.135	0.002	14.905	0.023	5.959	0.027
24	0.153	0.005	13.965	0.063	5.869	0.173
32	0.177	0.008	12.528	0.055	5.147	0.114
40	0.198	0.003	11.742	0.042	5.117	0.033
48	0.241	0.008	9.478	0.208	5.119	0.137
56	0.303	0.003	7.661	0.107	5.141	0.055
64	0.327	0.007	5.492	0.023	4.673	0.743
72	0.416	0.015	2.734	0.172	4.343	0.148
80	0.522	0.021	0.424	0.017	4.066	0.058
88	0.580	0.026	0.277	0.177	3.343	0.148

Variant 17(T:15°C, L:15.7 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.100	0.000	16.012	0.083	5.733	0.484
8	0.092	0.006	15.842	0.038	6.548	0.694
16	0.107	0.005	15.241	0.275	6.957	0.679
24	0.121	0.003	14.765	0.240	6.017	0.885
32	0.134	0.005	14.188	0.300	6.498	0.406
40	0.145	0.006	13.458	0.287	6.790	0.772
48	0.164	0.014	12.735	0.276	6.401	1.026
56	0.176	0.006	11.654	0.316	5.705	0.586
64	0.196	0.007	10.732	0.518	5.828	0.559
72	0.215	0.010	9.574	0.485	5.368	0.367
80	0.259	0.011	8.229	0.565	5.682	0.296
88	0.274	0.012	6.841	0.667	4.993	0.268
96	0.304	0.015	5.612	0.836	5.253	0.580

Variant 18(T:15°C, L:7.8 µmolm⁻²s⁻¹)

Time (h)	OD_{678} ±SD		N (mg l⁻¹) ±SD		P (mg l⁻¹) ±SD	
0	0.114	0.000	15.989	0.105	6.331	0.207
8	0.128	0.001	15.571	0.193	6.317	0.254
16	0.132	0.004	15.222	0.165	5.064	1.004
24	0.146	0.003	14.965	0.151	5.323	0.220
32	0.155	0.001	14.736	0.254	4.829	0.449
40	0.168	0.001	14.390	0.167	4.777	0.489
48	0.179	0.002	14.251	0.257	5.426	0.371
56	0.192	0.003	13.961	0.164	5.209	0.214
64	0.204	0.002	13.758	0.076	5.015	0.401
72	0.215	0.004	13.641	0.043	5.365	0.284
80	0.237	0.007	13.203	0.479	4.457	0.711
88	0.257	0.008	12.643	0.699	4.170	0.463
96	0.283	0.009	12.018	0.594	4.730	0.294
104	0.317	0.017	10.918	0.322	4.369	1.160

Variant 19(T:20^{0}C, L:78.3 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.090	0.000	16.178	0.000	5.655	0.432
8	0.122	0.011	14.672	0.923	4.827	0.730
16	0.222	0.011	12.475	0.951	4.378	0.765
24	0.305	0.021	8.936	2.049	3.327	0.476
32	0.428	0.030	4.752	1.696	2.702	0.211
40	0.609	0.032	1.338	0.860	2.961	0.548
48	0.759	0.016	1.063	0.704	3.019	0.751
56	0.843	0.023	0.723	0.578	2.465	0.527
64	0.902	0.022	0.420	0.398	4.637	0.098
72	0.949	0.021	0.193	0.319	4.276	0.265
80	0.982	0.020	0.055	0.049	3.975	0.262

Variant 20(T:20^{0}C, L:62.7 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.082	0.000	16.312	0.000	5.939	0.485
8	0.115	0.004	14.752	0.525	6.048	0.641
16	0.161	0.004	14.027	0.928	6.164	0.236
24	0.244	0.007	10.059	0.466	5.667	0.471
32	0.361	0.009	8.486	0.848	5.974	0.297
40	0.484	0.013	6.474	0.503	5.031	0.582
48	0.607	0.015	5.559	0.742	4.830	0.073
56	0.686	0.018	6.459	2.244	4.364	0.312
64	0.752	0.030	2.413	0.491	3.974	0.297
72	0.815	0.007	2.051	0.636	3.136	0.109
80	0.841	0.010	1.240	0.451	3.171	0.210

Variant 21(T:20^{0}C, L:47.0 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.101	0.000	15.942	0.093	6.254	0.362
8	0.152	0.002	13.508	0.116	5.170	0.831
16	0.204	0.005	11.191	0.231	5.501	0.687
24	0.297	0.006	8.507	0.260	5.124	0.467
32	0.440	0.004	4.456	0.286	4.761	0.709
40	0.581	0.011	1.253	0.180	4.681	0.484
48	0.690	0.002	0.515	0.205	4.273	0.376
56	0.787	0.004	0.026	0.006	3.240	0.353
64	0.812	0.013	0.050	0.020	2.093	0.472
72	0.840	0.010	-	-	1.252	0.338
80	0.861	0.006	-	-	0.530	0.415

Variant 22(T:20^{0}C, L:31.3 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.098	0.000	16.237	0.584	5.969	0.429
8	0.122	0.002	14.469	0.115	5.717	0.708
16	0.170	0.004	12.766	0.535	5.014	0.384
24	0.213	0.002	10.365	0.383	4.614	0.741
32	0.279	0.009	7.185	0.336	4.699	0.687
40	0.366	0.012	4.910	0.447	4.222	0.482
48	0.455	0.013	2.655	0.372	3.782	0.626
56	0.565	0.020	0.693	0.258	3.504	0.241
64	0.656	0.005	0.129	0.143	3.340	0.745
72	0.702	0.010	0.129	0.143	2.404	0.242
80	0.734	0.005	-	-	1.091	0.092

Light-Temperature experiment with *Scenedesmus subspicatus*

Variant 23(T:20°C, L:15.7 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.100	0.000	16.061	0.105	5.584	0.452
8	0.113	0.001	14.798	0.166	5.661	0.513
16	0.134	0.003	13.690	0.102	5.197	0.793
24	0.159	0.004	13.250	0.090	5.264	0.660
32	0.183	0.004	12.079	0.650	5.000	0.235
40	0.206	0.005	10.932	0.761	5.153	0.696
48	0.235	0.006	9.345	0.289	5.132	0.745
56	0.261	0.006	6.976	0.123	5.260	0.315
64	0.298	0.007	5.466	0.198	5.071	0.199
72	0.338	0.008	4.478	0.383	4.941	1.243
80	0.390	0.018	3.656	0.267	4.801	0.271
88	0.440	0.017				

Variant 24(T:20°C, L:7.8 µmolm^{-2}s^{-1})

Time (h)	OD_{678} ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
0	0.107	0.000	16.018	0.008	6.042	0.092
8	0.117	0.002	15.562	0.055	4.818	0.387
16	0.134	0.001	14.578	0.358	4.914	0.640
24	0.152	0.001	13.810	0.400	4.805	0.389
32	0.174	0.003	13.126	0.076	-	-
40	0.181	0.007	12.157	0.146	-	-
48	0.197	0.006	11.620	0.223	4.875	0.359
56	0.216	0.007	11.163	0.209	4.310	0.380
64	0.231	0.004	10.676	0.242	4.731	0.668
72	0.252	0.008	10.053	0.326	3.954	0.585
80	0.267	0.007	9.478	0.425	4.379	0.935
88	0.283	0.010	8.869	0.733	4.528	0.594
96	0.312	0.011	8.379	0.544	4.550	0.259
104	0.333	0.013	7.572	0.588	4.470	0.273

Light-Temperature experiment with *Scenedesmus subspicatus*

***Scenedesmus subspicatus*: Oxygen production (mg l^{-1}) ±SD**

Time (hrs)	variant 1		variant 2		variant 3		variant 4	
2	0.047062	0.001	0.044797	0.002	0.040989	0.005	0.027628	0.000
4	0.080232	0.001	0.083957	0.004	0.076720	0.005	0.046836	0.001
6	0.118506	0.001	0.124803	0.006	0.107979	0.007	0.066850	0.001
8	0.160278	0.001	0.168288	0.011	0.147343	0.009	0.092050	0.001

Time (hrs)	variant 5		variant 6		variant 7		variant 8	
2	0.016849	0.000	0.007902	0.002	0.074719158	0.009	0.066658507	0.004
4	0.032139	0.000	0.015245	0.003	0.132394	0.009	0.123408397	0.008
6	0.047076	0.001	0.023945	0.002	0.180801975	0.013	0.195264814	0.012
8	0.059018	0.000	0.026190	0.002	0.265467785	0.014	0.272887173	0.025

Time (hrs)	variant 9		variant 10		variant 11		variant 12	
2	0.06620	0.007	0.06704	0.019	0.04497	0.009	0.02656	0.004
4	0.11199	0.015	0.11921	0.004	0.06507	0.006	0.06322	0.003
6	0.18695	0.021	0.17263	0.020	0.13684	0.017	0.09373	0.004
8	0.24016	0.015	0.23040	0.030	0.17613	0.007	0.12713	0.007

Time (hrs)	variant 13		variant 14		variant 15		variant 16	
1	0.1924	0.011	0.1124	0.008	0.1204	0.014	0.1062	0.020
2	0.3874	0.041	0.3032	0.037	0.2240	0.011	0.2166	0.033
3	0.5380	0.040	0.4001	0.034	0.2687	0.026	0.3129	0.033
4	0.6996	0.048	0.6013	0.034	0.3680	0.013	0.3855	0.017
5	0.8821	0.081	0.7345	0.054	0.5009	0.011	0.4824	0.033
6	1.1337	0.086	0.8374	0.076	0.5729	0.088	0.5681	0.043
7	1.1849	0.098	1.0113	0.072	0.7432	0.041	0.6496	0.069
8	1.2943	0.064	1.1089	0.090	0.9148	0.074	0.8035	0.067

Time (hrs)	variant 17		variant 18		variant 19		variant 20	
1	0.0394	0.020	0.0217	0.010	0.195	0.033	0.127	0.006
2	0.0492	0.006	0.0226	0.004	0.316	0.053	0.285	0.022
3	0.0726	0.004	0.0382	0.003	0.449	0.049	0.380	0.012
4	0.1003	0.010	0.0550	0.002	0.589	0.047	0.471	0.018
5	0.1340	0.008	0.0782	0.010	0.699	0.053	0.562	0.016
6	0.1409	0.011	0.0879	0.006	0.870	0.049	0.644	0.016
7	0.1635	0.008	0.1058	0.007	0.977	0.116	0.780	0.019
8	0.1888	0.027	0.1207	0.006	1.159	0.106	0.859	0.020

Time (hrs)	variant 21		variant 22		variant 23		variant 24	
1	0.099	0.009	0.054	0.000	0.030	0.007	0.012	0.001
2	0.181	0.013	0.101	0.006	0.057	0.002	0.021	0.000
3	0.251	0.018	0.138	0.006	0.075	0.009	0.031	0.002
4	0.332	0.005	0.151	0.024	0.097	0.009	0.039	0.000
5	0.382	0.023	0.231	0.007	0.121	0.001	0.050	0.002
6	0.436	0.022	0.281	0.010	0.144	0.004	0.063	0.001
7	0.468	0.021	0.275	0.060	0.161	0.012	0.075	0.003
8	0.517	0.037	0.347	0.043	0.187	0.013	0.081	0.002

Data on effect of pH on nutrient presipitation

pH	DIC (mg l^{-1}) ±SD		N (mg l^{-1}) ±SD		P (mg l^{-1}) ±SD	
8.55	220.10	0.707	16.48	1.49	5.74	1.32
8.73	224.80	2.138	16.13	2.68	4.68	2.01
8.91	228.35	0.354	15.83	1.95	5.06	1.10
9.05	228.75	0.495	15.04	0.81	4.71	2.02
9.2	226.25	0.071	15.26	2.09	4.49	1.65
9.36	231.10	1.556	17.03	1.72	6.00	1.85
9.49	229.45	0.212	14.98	1.61	5.25	1.00
9.63	231.00	0.707	14.78	1.54	4.58	1.20
9.77	236.00	0.566	15.23	1.54	5.99	1.56
9.94	230.55	0.636	15.49	1.54	6.18	1.44
10.14	229.20	0.141	15.96	1.06	4.66	0.62
10.4	238.20	0.990	14.97	1.56	5.17	1.54
10.78	230.85	0.636	16.02	2.27	3.79	2.39
11.25	228.15	1.485	15.78	2.30	4.11	1.90
11.56	228.35	1.202	15.85	2.75	5.20	2.59
11.73	226.55	0.354	16.02	3.48	4.26	1.45
11.86	233.75	0.636	15.37	2.95	5.19	1.84
11.96	232.50	0.424	15.71	1.87	5.62	1.65

Data on algal respiration ($mgO_2mgDW\ min^{-1}$) ±SD

T(^{0}C)	*S.subspicatus*		*C.vulgaris*	
5	0.0024	0.0009	0.0013	0.0001
10	0.0050	0.0013	0.0033	0.0003
15	0.0051	0.0008	0.0044	0.0007
20	0.0086	0.0016	0.0068	0.0005

Light ($\mu molm^{-2}s^{-1}$)	*S.subspicatus*		*C.vulgaris*	
7.8	0.0011	0.0002	0.0035	0.0001
15.7	0.0024	0.0002	0.0044	0.0007
31.3	0.0051	0.0012	0.0052	0.0007
47.0	0.0057	0.0009	0.0073	0.0003
62.7	0.0084	0.0008	0.0090	0.0001
78.32	0.0114	0.0007	0.0101	0.0017

Survival experiment with *Chlorella vulgaris*

	OD_{678} ±SD	
Time (h)	week 0	
0	0.085	0.000
8	0.141	0.002
16	0.177	0.002
24	0.194	0.004

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD							
Time (h)	week 1		week 2		week 3		week 4	
0	0.084	0.000	0.081	0.000	0.086	0.000	0.080	0.000
8	0.105	0.002	0.106	0.004	0.106	0.004	0.106	0.014
16	0.118	0.005	0.138	0.003	0.131	0.008	0.127	0.015
24	0.157	0.017	0.181	0.006	0.161	0.018	0.145	0.021

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD							
Time (h)	week 5		week 6		week 7		week 8	
0	0.085	0.000	0.070	0.000	0.068	0.000	-	-
8	0.117	0.001	0.084	0.002	0.085	0.001	-	-
16	0.151	0.005	0.104	0.002	0.098	0.003	-	-
24	0.157	0.004	0.126	0.001	0.125	0.002	-	-

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD							
Time (h)	week 9		week 10		week 11		week 12	
0	-	-	0.078	0.000	0.058	0.000	0.057	0.000
8	-	-	0.091	0.001	0.082	0.002	0.076	0.002
16	-	-	0.111	0.002	0.097	0.003	0.094	0.003
24	-	-	0.158	0.005	0.109	0.003	0.112	0.002

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD							
Time (h)	week 13		week 14		week 15		week 16	
0	-	-	0.055	0.000	0.073	0.000	0.064	0.000
8	-	-	0.056	0.001	0.086	0.002	0.082	0.002
16	-	-	0.096	0.004	0.103	0.002	0.108	0.002
24	-	-	0.124	0.009	0.134	0.002	0.126	0.002

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD							
Time (h)	week 17		week 18		week 19		week 20	
0	0.071	0.000	0.069	0.000	0.071	0.000	0.071	0.000
8	0.081	0.001	0.089	0.004	0.090	0.001	0.082	0.001
16	0.098	0.001	0.123	0.003	0.107	0.006	0.102	0.001
24	0.129	0.001	0.130	0.001	0.128	0.001	0.128	0.000

***Chlorella vulgaris* group 1 (+4°C)**

	OD_{678} ±SD			
Time (h)	week 21		week 22	
0	0.069	0.000	0.061	0.000
8	0.081	0.001	0.076	0.002
16	0.097	0.001	0.092	0.001
24	0.137	0.006	0.123	0.002

Survival experiment with *Chlorella vulgaris*

	OD_{678} ±SD	
Time (h)	week 0	
0	0.085	0.000
8	0.141	0.002
16	0.177	0.002
24	0.194	0.004

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD							
Time (h)	week 1		week 2		week 3		week 4	
0	0.066	0.000	0.060	0.000	0.057	0.000	0.050	0.000
8	0.069	0.003	0.068	0.004	0.059	0.001	0.053	0.001
16	0.071	0.002	0.074	0.005	0.063	0.002	0.061	0.005
24	0.081	0.001	0.078	0.007	0.069	0.004	0.058	0.000

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD							
Time (h)	week 5		week 6		week 7		week 8	
0	0.057	0.000	0.061	0.000	0.045	0.000	-	-
8	0.064	0.002	0.067	0.002	0.049	0.001	-	-
16	0.071	0.004	0.073	0.002	0.059	0.004	-	-
24	0.081	0.002	0.086	0.001	0.077	0.008	-	-

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD							
Time (h)	week 9		week 10		week 11		week 12	
0	-	-	0.056	0.000	0.052	0.000	0.062	0.000
8	-	-	0.058	0.002	0.055	0.001	0.064	0.001
16	-	-	0.059	0.002	0.062	0.002	0.072	0.001
24	-	-	0.082	0.022	0.078	0.001	0.088	0.002

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD							
Time (h)	week 13		week 14		week 15		week 16	
0	-	-	0.040	0.000	0.050	0.000	0.050	0.000
8	-	-	0.049	0.002	0.053	0.002	0.054	0.001
16	-	-	0.047	0.003	0.053	0.001	0.060	0.002
24	-	-	0.056	0.004	0.056	0.002	0.067	0.001

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD							
Time (h)	week 17		week 18		week 19		week 20	
0	0.044	0.000	0.057	0.000	0.044	0.000	0.044	0.000
8	0.046	0.001	0.046	0.001	0.048	0.001	0.053	0.001
16	0.053	0.001	0.046	0.001	0.054	0.001	0.056	0.001
24	0.059	0.001	0.046	0.001	0.059	0.001	0.059	0.001

***Chlorella vulgaris* group 1 (-20°C)**

	OD_{678} ±SD			
Time (h)	week 21		week 22	
0	0.051	0.000	0.052	0.000
8	0.053	0.001	0.055	0.002
16	0.060	0.001	0.058	0.001
24	0.063	0.002	0.060	0.003

Survival experiment with *Scenedesmus subspicatus*

	OD_{678} ±SD	
Time (h)	**week 0**	
0	0.087	0.000
8	0.105	0.002
16	0.134	0.001
24	0.209	0.005

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD							
Time (h)	**week 1**		**week 2**		**week 3**		**week 4**	
0	0.092	0.000	0.084	0.000	0.084	0.000	0.080	0.000
8	0.101	0.003	0.099	0.002	0.098	0.001	0.090	0.001
16	0.133	0.006	0.136	0.003	0.122	0.003	0.103	0.003
24	0.187	0.013	0.174	0.014	0.158	0.003	0.141	0.005

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD							
Time (h)	**week 5**		**week 6**		**week 7**		**week 8**	
0	0.081	0.000	0.071	0.000	0.065	0.000	-	-
8	0.106	0.004	0.084	0.002	0.075	0.002	-	-
16	0.119	0.002	0.102	0.003	0.087	0.005	-	-
24	0.147	0.014	0.117	0.004	0.103	0.005	-	-

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD							
Time (h)	**week 9**		**week 10**		**week 11**		**week 12**	
0	-	-	0.075	0.000	0.065	0.000	0.065	0.000
8	-	-	0.077	0.001	0.069	0.001	0.071	0.002
16	-	-	0.090	0.001	0.084	0.002	0.081	0.001
24	-	-	0.131	0.002	0.114	0.004	0.100	0.001

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD							
Time (h)	**week 13**		**week 14**		**week 15**		**week 16**	
0	-	-	0.063	0.000	0.069	0.000	0.065	0.000
8	-	-	0.078	0.002	0.060	0.001	0.062	0.001
16	-	-	0.052	0.001	0.059	0.001	0.059	0.001
24	-	-	0.056	0.001	0.058	0.000	0.056	0.001

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD							
Time (h)	**week 17**		**week 18**		**week 19**		**week 20**	
0	0.070	0.000	0.066	0.000	0.070	0.000	0.070	0.000
8	0.067	0.001	0.051	0.001	0.067	0.002	0.052	0.000
16	0.059	0.001	0.052	0.002	0.062	0.003	0.047	0.001
24	0.050	0.000	0.050	0.000	0.051	0.000	0.050	0.000

***Scenedesmus subspicatus* group 1 (+4^{0}C)**

	OD_{678} ±SD			
Time (h)	**week 21**		**week 22**	
0	0.063	0.000	0.059	0.000
8	0.054	0.002	0.049	0.001
16	0.050	0.002	0.047	0.001
24	0.052	0.002	0.045	0.001

Survival experiment with *Scenedesmus subspicatus*

	OD_{678} ±SD	
Time (h)	week 0	
0	0.087	0.000
8	0.105	0.002
16	0.134	0.001
24	0.209	0.005

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD							
Time (h)	week 1		week 2		week 3		week 4	
0	0.085	0.000	0.080	0.000	0.070	0.000	0.068	0.000
8	0.087	0.002	0.082	0.002	0.069	0.001	0.071	0.001
16	0.086	0.001	0.072	0.002	0.066	0.002	0.076	0.001
24	0.073	0.004	0.079	0.000	0.065	0.002	0.069	0.001

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD							
Time (h)	week 5		week 6		week 7		week 8	
0	0.071	0.000	0.070	0.000	0.063	0.000	-	-
8	0.071	0.000	0.069	0.001	0.070	0.000	-	-
16	0.069	0.001	0.068	0.001	0.067	0.001	-	-
24	0.069	0.001	0.067	0.001	0.065	0.001	-	-

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD							
Time (h)	week 9		week 10		week 11		week 12	
0	-	-	0.083	0.000	0.081	0.000	0.070	0.000
8	-	-	0.085	0.001	0.076	0.001	0.071	0.002
16	-	-	0.086	0.001	0.073	0.001	0.069	0.001
24	-	-	0.084	0.000	0.069	0.001	0.068	0.001

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD							
Time (h)	week 13		week 14		week 15		week 16	
0	-	-	0.058	0.000	0.074	0.000	0.072	0.000
8	-	-	0.065	0.002	0.068	0.001	0.069	0.001
16	-	-	0.061	0.001	0.067	0.000	0.066	0.001
24	-	-	0.058	0.002	0.056	0.001	0.057	0.001

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD							
Time (h)	week 17		week 18		week 19		week 20	
0	0.071	0.000	0.074	0.000	0.070	0.000	0.068	0.000
8	0.069	0.001	0.067	0.001	0.068	0.001	0.066	0.001
16	0.066	0.001	0.060	0.003	0.062	0.001	0.060	0.001
24	0.060	0.001	0.056	0.001	0.056	0.001	0.055	0.001

Scenedesmus subspicatus group 1 (-20°C)

	OD_{678} ±SD			
Time (h)	week 21		week 22	
0	0.072	0.000	0.069	0.000
8	0.066	0.001	0.064	0.001
16	0.060	0.001	0.055	0.001
24	0.056	0.001	0.054	0.001

Data for Survival experiment with *Chlorella vulgaris*

***Chlorella vulgaris*: Group 1(+4°C)**

Time	DW ±SD (mgl^{-1})		Chl a ±SD (mgl^{-1})		P ±SD (mgC mg chla h^{-1})		cells (x 10^{9}) ±SD (l^{-1})	
week 0	21.0	1.0	0.320	0.004	0.117	0.012	13.0	0.8
week 1	17.7	2.3	0.329	0.040	0.063	0.007	11.7	1.1
week 2	16.0	1.7	0.299	0.001	0.050	0.006	11.9	0.6
week 3	17.7	2.3	0.232	0.002	0.051	0.007	9.5	0.7
week 4	19.0	1.0	0.267	0.003	0.032	0.008	8.9	0.7
week 5	18.8	3.5	0.305	0.013	0.026	0.003	9.4	0.8
week 6	14.0	1.0	0.274	0.004	0.040	0.002	9.0	1.7
week 7	14.8	0.6	0.272	0.035	0.051	0.005	8.4	0.4
week 8	-	-	-	-	-	-	-	-
week 9	-	-	-	-	-	-	-	-
week 10	13.1	3.1	0.289	0.025	0.071	0.010	7.1	1.0
week 11	15.3	3.5	0.321	0.009	0.105	0.021	9.3	1.2
week 12	13.7	1.2	0.261	0.004	0.079	0.009	8.4	0.9
week 13	-	-	-	-	-	-	-	-
week 14	14.5	3.3	0.271	0.011	0.094	0.006	6.5	0.8
week 15	18.6	3.5	0.294	0.006	0.084	0.010	4.0	0.4
week 16	16.3	0.6	0.276	0.005	0.089	0.002	10.0	0.9
week 17	16.7	1.5	0.230	0.002	0.087	0.005	11.0	0.1
week 18	11.1	3.6	0.224	0.013	0.055	0.003	13.5	1.4
week 19	18.5	3.1	0.140	0.001	0.096	0.001	8.1	2.5
week 20	19.7	1.3	0.268	0.013	0.062	0.002	13.6	1.9
week 21	15.1	3.7	0.294	0.012	0.166	0.010	11.7	1.7
week 22	21.7	1.4	0.254	0.004	0.085	0.000	11.7	0.3

***Chlorella vulgaris*: Group 2 (-20°C)**

Time	DW ±SD (mgl^{-1})		Chl a ±SD (mgl^{-1})		P ±SD (mgC mg chla h^{-1})		cells (x 10^{9}) ±SD (l^{-1})	
week 0	21.0	1.0	0.32	0.004	0.117	0.012	13.0	0.8
week 1	4.3	2.1	0.173	0.003	0.037	0.009	2.6	0.3
week 2	4.0	2.6	0.209	0.002	0.021	0.002	3.1	0.5
week 3	4.7	2.1	0.054	0.001	0.040	0.002	1.1	0.1
week 4	3.7	1.5	-0.008	0.003	0.050	0.007	1.0	0.0
week 5	4.8	0.3	0.008	0.021	0.029	0.004	1.8	0.2
week 6	4.7	1.2	0.005	0.001	0.041	0.005	1.5	0.1
week 7	-0.5	4.8	0.014	0.008	0.029	0.004	1.6	0.1
week 8	-	-	-	-	-	-	-	-
week 9	-	-	-	-	-	-	-	-
week 10	3.5	3.9	-0.026	0.005	0.048	0.005	1.1	0.1
week 11	4.7	1.2	0.012	0.013	0.074	0.010	1.7	0.1
week 12	5.0	1.0	0.013	0.002	0.048	0.004	1.0	0.1
week 13	-	-	-	-	-	-	-	-
week 14	4.7	1.2	-0.022	0.002	0.067	0.007	0.8	0.3
week 15	1.9	0.5	0.009	0.000	0.068	0.002	0.5	0.1
week 16	2.0	1.7	-0.002	0.005	0.066	0.005	1.8	0.2
week 17	1.3	0.6	-0.019	0.002	0.053	0.002	1.8	0.2
week 18	1.8	2.8	-0.044	0.002	0.025	0.001	2.9	0.4
week 19	-1.2	2.5	-0.037	0.002	0.060	0.002	0.7	0.4
week 20	5.9	1.6	-0.114	0.006	0.066	0.001	1.1	0.1
week 21	2.5	1.6	-0.022	0.003	0.060	0.006	2.8	0.1
week 22	4.8	0.9	-0.027	0.001	0.059	0.004	1.8	0.5

Data for Survival experiment with *Scenedesmus subspicatus*

***Scenedesmus subspicatus*: Group 1(+4°C)**

Time	DW ±SD (mgl^{-1})		Chl a ±SD (mgl^{-1})		P ±SD (mgC mg chla h^{-1})		cells (x 10^{9}) ±SD (l^{-1})	
week 0	36.3	3.2	0.406	0.003	0.160	0.013	3.1	0.2
week 1	35.3	0.6	0.400	0.016	0.058	0.009	3.6	0.3
week 2	33.7	0.6	0.395	0.022	0.057	0.003	3.2	0.6
week 3	28.7	2.1	0.329	0.010	0.071	0.002	2.3	0.2
week 4	26.0	1.7	0.331	0.021	0.042	0.007	3.5	0.1
week 5	27.4	2.1	0.289	0.002	0.087	0.006	2.8	0.4
week 6	22.7	2.1	0.271	0.002	0.095	0.009	3.2	0.3
week 7	17.9	4.4	0.265	0.030	0.049	0.002	2.8	0.7
week 8	-	-	-	-	-	-	-	-
week 9	-	-	-	-	-	-	-	-
week 10	18.5	1.5	0.217	0.004	0.063	0.008	2.0	0.5
week 11	14.0	1.7	0.185	0.006	0.142	0.016	2.2	0.5
week 12	9.7	2.1	0.145	0.005	0.067	0.006	1.7	0.2
week 13	-	-	-	-	-	-	-	-
week 14	6.2	1.2	-0.072	0.001	0.068	0.004	1.0	0.2
week 15	8.2	3.6	-0.073	0.007	0.065	0.005	0.6	0.1
week 16	4.7	1.5	-0.072	0.004	0.061	0.003	0.7	0.1
week 17	2.0	2.6	-0.082	0.001	0.059	0.004	0.4	0.0
week 18	-2.3	3.2	-0.076	0.029	0.032	0.001	0.3	0.1
week 19	-1.5	3.5	-0.102	0.000	0.044	0.002	0.3	0.1
week 20	-3.6	1.4	-0.109	0.003	0.075	0.003	0.5	0.1
week 21	0.7	1.7	-0.097	0.003	0.017	0.002	0.4	0.0
week 22	-0.1	3.5	-0.057	0.010	0.042	0.001	1.7	0.3

***Scenedesmus subspicatus*: Group 2(-20°C)**

Time	DW ±SD (mgl^{-1})		Chl a ±SD (mgl^{-1})		P ±SD (mgC mg chla h^{-1})		cells (x 10^{9}) ±SD (l^{-1})	
week 0	36.33	3.21	0.406	0.003	-	-	-	-
week 1	-0.67	2.08	-0.092	0.001	-	-	-	-
week 2	2.00	2.00	-0.094	0.002	-	-	-	-
week 3	0.33	0.58	-0.062	0.001	-	-	-	-
week 4	0.67	1.15	-0.073	0.002	-	-	-	-
week 5	-1.00	2.60	-0.055	0.002	-	-	-	-
week 6	1.00	1.00	-0.071	0.002	-	-	-	-
week 7	-2.19	0.39	-0.076	0.001	-	-	-	-
week 8	-	-	-	-	-	-	-	-
week 9	-	-	-	-	-	-	-	-
week 10	-2.30	3.75	-0.073	0.003	-	-	-	-
week 11	-0.67	2.31	-0.075	0.001	-	-	-	-
week 12	-2.67	2.31	-0.081	0.001	-	-	-	-
week 13	-	-	-	-	-	-	-	-
week 14	-3.33	1.15	-0.053	0.003	-	-	-	-
week 15	1.00	2.00	-0.063	0.001	-	-	-	-
week 16	1.00	3.61	-0.071	0.002	-	-	-	-
week 17	0.67	1.53	-0.076	0.002	-	-	-	-
week 18	-5.37	3.01	-0.089	0.001	-	-	-	-
week 19	-1.91	1.81	-0.090	0.001	-	-	-	-
week 20	-0.83	3.31	-0.080	0.001	-	-	-	-
week 21	-1.00	1.32	-0.090	0.001	-	-	-	-
week 22	-2.67	4.37	-0.082	0.002	-	-	-	-