THE CONSTRUCTION AND EVALUATION OF A NOVEL TUBULAR PHOTOBIOREACTOR AT A SMALL PILOT PLANT SCALE.

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DECLARATION

I, Kutama Makonde hereby declare that the construction and evaluation of a novel tubular photobioreactor at a small pilot plant scale is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. The thesis has not been submitted or will not be submitted to any other university or any institution for the award of a degree.

Signed (author) Date

Signed (supervisor) Date

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For my wonderful family,

whose love, humility,

and perseverance shows

no boundaries

ABSTRACT

The mass production of algae for commercial purposes has predominately been carried out in open ponds systems. However, open ponds systems have a number of disadvantages such as poor light utilization, requirement for large areas of land and high risks of contamination. On the other hand, photobioreactors have attracted much interest because they allow a better control of the cultivation conditions than open systems. With photobioreactors, higher biomass productivities are obtained and contamination can be easily prevented. Photobioreactors can also be engineered to manipulate the light and dark photosynthetic reactions thus enhancing biomass productivity.

The main objective of this study was to construct a novel tubular photobioreactor which had the ability to expose the cultured alga to light and dark phases with the aim of optimizing the algal biomass production.

A novel tubular photobioreactor with the ability to manipulate the cultured alga's light and dark photosynthetic reactions was constructed in this study. The alga *Spirulina platensis* was chosen as the test organism in this novel tubular photobioreactor due to a number of reasons such as its globally socioeconomic importance, its tolerance of higher pH and temperature values which makes it almost impossible to contaminate. The cultivation process of *Spirulina* in the photobioreactor was investigated through alternating light and dark cycles in an attempt to increase the photosynthetic efficiency of the culture. The effect of different light intensities on the growth of *Spirulina* in the novel tubular photobioreactor was investigated and it was found that the best light condition that favored higher biomass formation was at 600 μ mol m^{-2} s^{-1}. Five different light/ dark ratios were evaluated at a light intensity of 600 μ mol m^{-2} s^{-1} during a batch mode of operation of the novel tubular photobioreactor. The light/ dark ratio of 1:0.25 was found to be the best ratio because it gave the highest biomass in the shortest period of time when compared to the other ratios used. These results seem to suggest that longer light cycle relative to dark cycle results in higher biomass production. The ratio of 1:0.25 was then used to operate the novel tubular photobioreactor in a continuous mode. A maximum biomass productivity of 25 g/m^2/day was achieved which corresponded to a net photosynthetic

efficiency of 5.7 %. This result was found to be higher than what most photobioreactors could achieve but it was 2.8 g/m^2/day lower than the highest ever reported productivity in a photobioreactor when *Spirulina* is cultivated. The 2.8 g/m^2/day lower was attributed to the different materials used in the construction of these two photobioreactors. The photobioreactor which achieved 27.8 g/m^2/day was made up of a clear glass whereas the novel tubular photobioreactor was made up of a PVC tubing. PVC tubes tend to change from clear to a milky colour after a certain period when it is used at higher temperature and pH values hence blocks a certain amount of light. Therefore the main recommendation in this study is to use a PVC tubing with a longer life span when used at a higher temperature and pH values.

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CHAPTER 1

1. INTRODUCTION AND OVERVIEW

1.1 General background of the study

Algae are simple plants without roots, stems and leaves and they contain chlorophyll (Grobbelaar 1982; Anderson, Jennie & Angela 1996). They are of great interest to biologists because all algae (unicellular or multicellular) are complete organisms capable of photosynthesis and the production of a multitude of compounds which make up the cell (Harun, Singh, Forde & Danquah 2009). Algae occur in great abundance in oceans, seas, salt lakes, fresh water lakes, ponds and streams and can be found in many places where there is moisture, carbon dioxide and light. There are many different kinds of algae and they are classified according to the following characteristics (South & Whittick 1987; Pelczar, Chan & Krieg 2010):

(1) Nature and properties of pigments

(2) Nature of reserve and storage products

(3) Type and insertion (point of attachment) and morphology of flagella

(4) Chemical composition and physical features of the cell wall

(5) Morphology and characteristics of cells and thalli.

What makes algae important to biotechnologists is the fact that through the process of photosynthesis (in the presence of light, carbon dioxide and water) they are able to produce algal biomass (Collet, Hélias, Lardon, Ras, Goy & Steyer 2010; Grobbelaar 2000). This algal biomass can be used for various applications such as:

(1) Animal and human nutrition/feed

(2) Extraction of health products for pharmaceutical formulations/ concoctions and

(3) The production of energy through products such biofuel and the combustible dried biomass hence providing an alternative means of renewable energy production (Moreno-Garrido 2008; González-Fernández, Molinuevo-Salces & García-González 2010).

Although sunlight serves as sole source of energy for algal growth, the algae are unable to utilize energy from the sunlight to their full potential (Xue, Su & Cong 2010). As a result only 4 to 6% (Anderson 2005) of the energy absorbed from the sunlight is directed towards the production of biomass. This serves as a challenge for biotechnological applications that rely on sunlight for algal biomass production.

In an effort to counter the challenge presented above, some algal producers have resorted to the use of photobioreactors to maximize sunlight exposure (Hsieh & Wu 2009; Rosello Sastre, Csögör, Perner-Nochta, Fleck-Schneider & Posten 2007). This has led to the increase in algal biomass turnover in various algal producing countries such as Spain, Israel and USA (ugwu & Aoyagi 2008; Celekli & Yavuzatmaca 2009; Masojídek, Torzillo, Sven & Brian 2008).

Although light is a major component required for algal biomass formation, it is important to remember that photosynthesis undergoes a dark phase for other critical metabolic reactions to occur. Bearing that in mind it has been demonstrated that the use of “dark/light” cycles (choosing the right periods of illumination and darkness) results in a higher biomass yield when compared to continuous light (Grobbelaar 1991), therefore this project focused on the development of a novel tubular photobioreactor that was later used to evaluate the effect of different dark/light cycle regimens on the biomass production of *Spirulina.*

In this study a tubular photobioreactor was constructed and operated at a small pilot plant scale both indoors (laboratory) and outdoors. The effect of the dark/light cycles on *Spirulina* biomass production was evaluated using artificial (commercial lights in the laboratory) and natural (sunlight outside the laboratory) light sources. The alga *Spirulina* was chosen as the test organism for the novel tubular photobioreactor due to the fact that it can easily be cultivated, requires high pH and temperature which inhibits the growth of most microalgae, can be easily harvested and lastly for its ever increasing demand on the international market. The

photobioreactor was operated as a batch and continuous system. The continuous system was achieved through the incorporation of a turbidostat which maintained a constant cell density of *Spirulina* by harvesting cells and adding fresh growth medium. Dry cell weight as well as turbidity was used to monitor cell growth (in the novel tubular photobioreactor) with respect to biomass production or growth dynamics in both systems. These parameters were followed continuously and correlated to the different dark/light cycle regimes.

1.2 Study aim and objectives

Aim:

The aim of this project was to develop a novel tubular photobioreactor and use it to optimize the production of *Spirulina* biomass.

Objectives:

1. To construct the novel tubular photobioreactor.
2. To select an alga (*Spirulina* as test algal species) that can be easily cultivated in a photobioreactor.
3. To determine at bench scale, the optimal growth conditions of *Spirulina* with regards to pH, temperature, growth medium, light and aeration.
4. To grow *Spirulina* in the photobioreactor under varying conditions of light /dark cycles using both artificial and natural light.
5. To operate the photobioreactor growing *Spirulina* in a continuous system in both artificial and natural light
6. To determine the operating conditions for an optimal *Spirulina* biomass production in the photobioreactor.

1.3 Significance of the study

Algal biomass from various algal species has widely been investigated for its potential application in pharmaceuticals (vitamins and nutritional supplements), cosmetics (fine chemicals), food industry (pigments, proteins and carbohydrates) and as a source of green energy (biofuel). However, in order to achieve these applications, an efficient culturing method/system which favours an increase in the algal optimal biomass production is required. In this project a tubular photobioreactor was developed and used to increase the optimal biomass production of *Spirulina*.

CHAPTER 2

2. Literature Review

2.1 History/ importance of algal production

Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to that of plants, however their simple cellular structure makes them more efficient in converting solar energy into biomass (Radmer 1996; Carlsson, Van Beilen, Möller & Clayton 2007). Micro-algae constitute a polyphyletic and highly diverse group of prokaryotic (two divisions) and eukaryotic (nine divisions) microorganisms (South & Whittick 1987; Carlsson *et al*. 2007). The classification of micro-algae is based on various properties such as pigmentation, the organization of photosynthetic membranes and other morphological features (size, shape, motility etc.) (Radmer 1996; South & Whittick 1987).

Micro-algae have contributed to the social and economic growth of various countries worldwide. These microorganisms have served as a source of medicine, food and nutrition as well as bio-energy (green energy). The most studied micro-algae have been: *Chlorophyceae* (green algae) as a source of food (e.g. *Dunaliella*), *Bacillariophyceae* (such as the diatoms) as a source of oils and abrasives and chrysolaminarin, *Chrysophyceae* (such as the golden algae) as a source of lipids and carbohydrates and last but not least the *Cyanophyceae* (blue-green algae) as a source of food (Carlsson *et al*. 2007; Gal & Ulber 2005).

Spirulina platensis, a cyanobacterium has been consumed by humans for a long time. This micro-alga has been shown to be a rich source of protein (or amino acids), carbohydrates, vitamins and trace elements such as zinc, iron and calcium. Due to *Spirulina*'s high nutritional content and pigmentation, its production has been expanded by the use of modern biotechnological methods (Richmond 2004). The annual world production of *Spirulina* in the year 2004 was estimated to be 6000 tons (Botha 2004). However, the demand for this particular micro-alga has increased due to its application in industries other than the food industry. For instance, *Spirulina* has been used in the production of fine chemicals for clinical

diagnosis, biological research, and cosmetics (Richmond 2004). Furthermore the therapeutic and health effects of *Spirulina* are gaining popularity within the pharmaceutical and nutraceutical industries. Given this, it is anticipated that *Spirulina* will continue to act as one of the most important cyanobacterial species within the biotechnology industry (Belay 2002). Therefore, the commercial production of *Spirulina* needs to be improved in order to meet its increasing demand.

2.2. Different ways in which microalgae can be cultured

At present, the commercial production of phototrophic microbial biomass is limited to a few micro-algal species. These micro-algal species have been cultivated in open pond systems (Benemann 2009; Richmond 2004). The open pond systems are usually manipulated to enable the selection of the desired algal species which then out- compete the growth of the undesired micro-algae (Carlsson *et al*. 2007). Most microalgae cultures cannot be maintained long enough in open pond systems due to contamination by fungi, bacteria, and protozoa as well as the risk posed by other microalgae whose growth rate could be enhanced by the ambient conditions (Sastre, Rosello, Csögör, Perner-Nochta, Pascale & Posten 2007; Benemann 2009; Pulz 2001).

In contrast to open pond systems, photobioreactors (PBR) offer a means of shielding the algal culture form the outside environment. This in turn offers the micro-algae cultivated in PBR some form of protection against most of the disadvantages associated with open pond systems. Furthermore PBR provide a better means of controlling the environment within which the micro-algae are growing thus ensuring the dominance of the desired species (Benemann 2009; Pulz 2001). Taking all this into account, PBRs allow for the exploitation of potential micro-algae (of more than 50 000 species) for economic interest (Richmond 2004).

2.2.1 Open system

Cultivation of algae in open systems has been extensively studied and predominantely used by industries for the mass production of algae. Open systems have been categorized into natural waters (lakes, lagoons, ponds) and artificial ponds or containers (Chaumont 1993; Borowitzka 1999; Pulz 2001). In the artificial open system also referred to as open ponds, the water is typically kept in motion/mixed by paddlewheels (Figure 1) or rotating structures (Chaumont 1993), and as well as appropriately designed guides (Ugwu *et al*. 2008). The nutrients are usually provided through runoff water from nearby land areas (natural waters) or in a controlled manner (artificial ponds) (Gallagher 2010; Carlsson *et al*. 2007). One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems (Ugwu *et al*. 2008).

Figure 1: Cultivation of algae in an open pond system (Alok 2008).

2.2.2. Closed system

Closed systems also referred to as photobioreactors (Figure 2) are vessels in which algae are cultivated (Carlsson *et al*. 2007). Algal culture systems can be illuminated by artificial light, solar light or by both (Ugwu *et al*. 2008; Demirbas 2010).

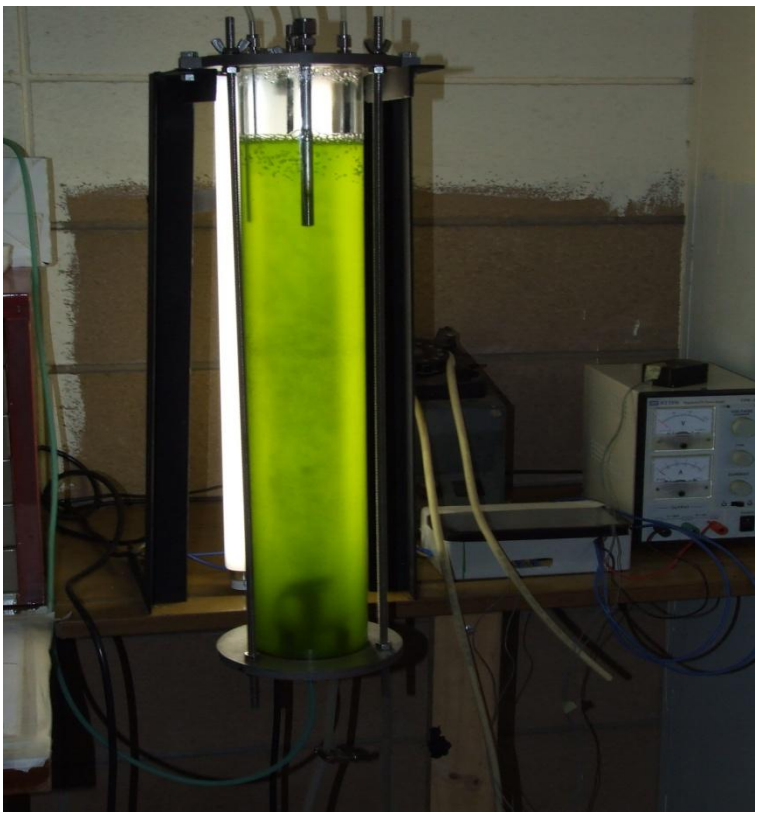

Figure 2: Cultivation of algae in a photobioreactor.

Generally, laboratory-scale photobioreactors can be naturally illuminated using sunlight or artificially illuminated (either internally or externally) using photosynthetic light. The micro-algae grown in such closed systems are provided with the necessary nutrients and sometimes CO_2 in a controlled manner with allowance for degassing (expulsion of oxygen) (Carlsson *et al*. 2007). Some of these photobioreactors include bubble column, airlift column, stirred-tank, helical tubular, conical, torus, and seaweed type photobioreactors (Pulz 2001).

Photobioreactors are flexible and portable hence making them more desirable to use in various settings. For example tempering (Improve the consistency or resiliency of a substance) could simply be achieved by placing a photobioreactor in a room with a constant temperature. Thus tempering is limited to compact photobioreactors, however, large scale outdoor systems such as tubular photobioreactors cannot be easily tempered without high technical efforts (Ugwu *et al*. 2008).

2.2.3. Challenges associated with the commercial production of algae

Algae have been commercially grown using open pond systems with the exception of a few countries (United States of America and German) (Borowitzka 1999) in which closed systems have been introduced. Open pond systems in comparison to closed systems have been preferred due to a number of reasons (Table 1) with the most important ones being the cost of construction as well as maintenance.

Table 1: Comparison of open and closed system (Grobbelaar 2008)

Parameters	**Open ponds**	**Closed system (PBR systems)**
Contamination risk	High	Low
Water losses	High	Low
Carbon dioxide losses	High	Almost none
Reproducibility of production	Variable but consistent over time	Possible within certain tolerances
Process control	Complicated	Less
Standardization	Difficult	Possible
Weather dependence	High	Less because it is protected
Maintenance	Easy	Difficult
Construction costs	Low	High
Biomass concentration at harvesting	Low	High
Overheating problems	Low	High
Dissolved oxygen concentration	Low	High

However open pond systems face more serious challenges such as poor light penetration, evaporation, diffusion of CO_2 into the atmosphere which impact on the efficiency and productivity of the algal biomass and the requirement for large areas of land (Cohen & Arad 1989; Converti, Lodi, Del Borghi & Solisio 2006a; Ugwu *et al*. 2008; Pulz 2001; Chaumont 1993). In addition, open cultivation systems are faced with inefficient stirring mechanisms that often result in very poor mass transfer rates resulting in low biomass productivity (Carlsson *et al*. 2007). Contamination in open systems has also restricted the commercial production of algae in such systems to only those organisms that can grow under extreme conditions. Currently only *Dunaliella* (high salinity tolerance), *Spirulina* (high alkalinity tolerance) and *Chlorella* (high nutrient tolerance) have been successfully grown commercially in open pond systems. In order

to overcome these problems, the commercial production of algae in closed systems (photobioreactors) has been suggested (Converti *et al*. 2006a). But the challenge associated with such a suggestion lies in the costs.

Photobioreactors have attracted much interest because they allow a better control of the cultivation conditions than open systems (Cohen & Arad 1989). With photobioreactors, higher biomass productivities are obtained and contamination can be easily prevented (Ugwu *et al*. 2008; Vonshak & Richmond 1988). Different types of photobioreactors such as flat plate, vertical column, internally illuminated and tubular are available but of interest to this study is the tubular photobioreactor.

2.3. Tubular photobioreactor

Tubular photobioreactors in relation to other photobioreactors are more suited for the commercial production of algae due to their large illumination surface area (Ugwu & Aoyagi 2008). These reactors are designed in a way that they can enable the utilization of both artificial and natural light. Light plays a critical role in algal biomass formation; therefore tubular photobioreactors consist of tubes that have been made from transparent material such as glass or plastic (polypropylene acrylic or polyvinylchloride) arranged in various ways (straight, coiled or looped) aimed at maximizing sunlight capture (Briassoulis, Panagakis, Chionidis, Tzenos, Lalos, Tsinos, Berberidis & Jacobsen 2010; Ugwu *et al*. 2008).

Various forms of tubular photobioreactors have been proposed, some of which include horizontal straight tubes connected by U-bends, α-type photobioreactor (Miyamoto, Wable & Benemann 1988) and the flat plate photobioreactors (Ugwu *et al*. 2008; Lee 2001). In these reactors the culture is circulated with the use of a pump hence enabling the mixing of the algal cultures (Ugwu *et al*. 2008). Tubular photobioreactors also have some disadvantages although these are mainly design specific (Ugwu *et al*. 2008).

The main disadvantages associated with tubular photobioreactors revolve around their high light energy requirements, cleaning and low efficiency in terms of mass production per unit of space. In addition the cultured algae is faced with undesirable hydrodynamic stress due to the flow characteristics (e.g. turbulent flow, pump type) and is design specific (Borowitzka 1999). Furthermore the operational difficulties affiliated with these photobioreactors may include: attachment of algae to the walls of the tubes thus blocking the light and high oxygen build up that may inhibit photosynthesis (Briassoulis *et al*. 2010).

2.4. Photosynthesis

Photosynthesis is a physico-chemical process by which light energy is used to drive the synthesis of organic compounds in organisms with chloroplasts. The net reaction for photosynthesis can be summed up as follows:

Carbon dioxide + Water + Light energy → Glucose + Oxygen

Photosynthesis is divided into two main reactions/processes (figure 3) namely light reactions (light dependent) and dark reactions (light independent) (Stevenson, Bothwell & Lowe 1996).

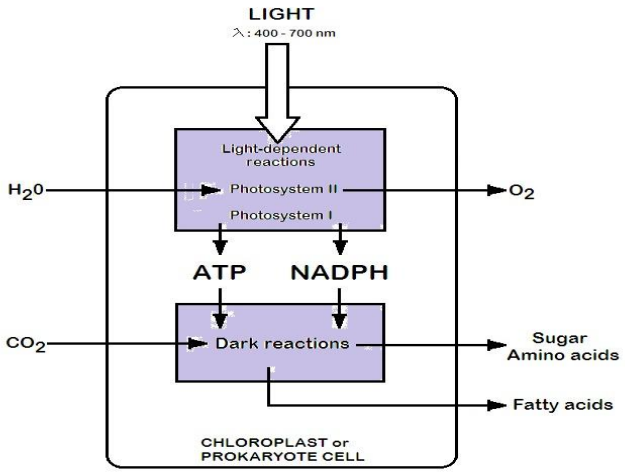

Figure 3: Light dependent and dark reactions in the photosynthetic process (Sigee 2005).

2.4.1. Light reactions

Light reactions take place in a thylakoid membrane. The reactions involve the capture of light energy and its transfer to adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrite (NADPH). Light reactions take place in Photosystem I (PS I) which is associated with the conversion of NADP to NADPH and Photosystem II (PS II) responsible for the splitting of water molecule (McDonald 2003; Stevenson *et al*. 1996). In organisms that carry out oxygenic photosynthesis (eukaryotic and prokaryotic algae), water is split as part of the reaction, releasing oxygen and making available electrons which convert $NADP^+$ to NADPH. Although both photosystems are required for complete photosynthesis, they are physically distinct and also have independent requirement for photons (Sigee 2005). Light reactions are temperature-independent since they are photochemical in nature and are not driven by enzymatic processes.

2.4.2. Dark reactions of photosynthesis

Dark reactions which occur in the dark a few hundredths of a second following the light reactions, are linked to the fixation of carbon dioxide. These reactions take place in the space between thylakoid membranes and utilize the energy and reducing power of ATP and NADPH to reduce CO_2 to hexose (6 carbon molecule). Dark reactions generate a variety of sugars, amino and fatty acids (Sigee 2005; McDonald 2003; Stevenson *et a*l, 1996).

2.5. Light/dark cycles and photosynthetic efficiency in photobioreactors

Photobioreactors should be designed in such a way so as to optimize efficient light exposure. The efficiency of light utilization is very important because light energy has been reported as a critical growth limiting factor during the operation of photobioreactors (Janssen, De Winter, Tramper, Mur, Snel & Wijffels 2000; Janssen, Slenders, Tramper, Mur & Wijffels 2001). Most photobioreactors, especially air-lift photobioreactors, do not entirely consist of an absolute dark region. Inside an air lift photobioreactor algae are exposed to high light intensity close to the reactor surface where the lights have been placed. As the light passes through the photobioreactor, its intensity is reduced in proportion to the density of the algal culture. Therefore the area within the photobioreactor that receives less light is classified as the “dark zone” where the dark cycle is said to occur (Grima, Fern´andez, Camacho & Rubio 2000; Janssen

et al. 2001). Less light does not mean absolute darkness. However in order to achieve absolute darkness these systems have been incorporated with timers that control the duration of light exposure (Janssen *et al*. 2001). This has enabled the assessment of the real light/dark cycles in such photobioreactors. On the other hand algae have been cultivated in tubular photobioreactors. The illumination of algal cultures within tubular photobioreactors is interrupted by an absolute dark region(s) that is accomplished by covering (painting or using foil) the tube at different intervals (Grobbelaar 1994). These intervals of complete light and darkness within the tubular photobioreactors have also allowed for the evaluation of light/dark cycles in such systems (Qiang, Zarmi & Richmond 1998).

Taking all that has been mentioned into account, the alteration between light and darkness is critical in the enhancement of photosynthetic efficiency which is dependent on the design of the photobioreactor (Terry 1986). The optimal alteration between light and darkness greatly enhances productivity/photosynthetic efficiency (Grobbelaar 1991; Terry 1986). Productivity is influenced by the frequency of the light/dark cycles (Grobbelaar 1989). Grobbelaar (1989) categorized the frequency fluctuations as high (>10 Hz), medium (seconds to minutes) and low (hours to days). Of these three, high frequency fluctuation has been reported to increase the rate of photosynthetic efficiency (Terry 1986; Grobbelaar 1989; Grobbelaar, Nedbal & Tichý 1996a) whereas low frequency fluctuation has been reported to influence algal cell division during photosynthesis as well as their circadian rhythm activity. On the other hand, according to Grobbelaar (1989) medium frequency fluctuation is associated with turbulence within the algal culture. Although there is not enough data on medium frequency fluctuations, Grobbelaar (1989) seems to suggest that turbulence may influence photosynthetic efficiency.

While there is tangible evidence that the light/dark cycle greatly enhances productivity with increasing frequency of the light/dark cycle, there is still a debate over which of the above frequencies is needed to achieve increased productivity. Kok (1953) demonstrated that *Chlorella pyrenoidosa* required a low ratio of light/dark cycle, where the dark cycle is precisely ten times more than the light cycle for the efficient utilization of light during photosynthesis for this particular organism. Contrary to the above, Grobbelaar *et al*. (1996a) reported that a longer

dark period relative to the preceding light period does not necessary lead to higher productivity. The contradiction in this issue could have resulted from the different experimental set-up such as the utilization of different algal strains (Grobbelaar *et al*. 1996a; Terry 1986; Kok 1953), acclimated (get used to a certain climate) algae as well as the use of a mixed culture (Grobbelaar *et al*. 1996a). Algal reports have also indicated that below a certain light/dark frequency the rate of photosynthesis and culture productivity declines. The frequency at which this happens is referred to as the critical value. Furthermore, the critical value has been reported to be culture-specific and can be determined by subjecting the cells to different cycle frequencies until a decline in productivity is observed (Terry 1986).

Photosynthesis results in more efficient productivity when the flash cycle frequency is identical to the turnover rate of electrons in the photosystem II of the photosynthetic apparatus (Grima *et al*. 2000). However, this may be culture specific and may also depend on how the culture was acclimatized. At optimal cell density (i.e. which results in highest productivity per irradiated area), cells are exposed to relatively short flashes of light followed by a relatively long period of darkness; the higher the frequency of this light/dark cycle therefore, the more efficiently light may be utilized for photosynthesis (Grobbelaar *et al*. 1996a; Janssen *et al*. 2000; Janssen 2002).

2.6. Operation modes of tubular photobioreactors

Algae can be grown in tubular photobioreactors following batch, fed-batch and continuous operation modes. All these operation modes rely on the algal growth curve (figure 4).

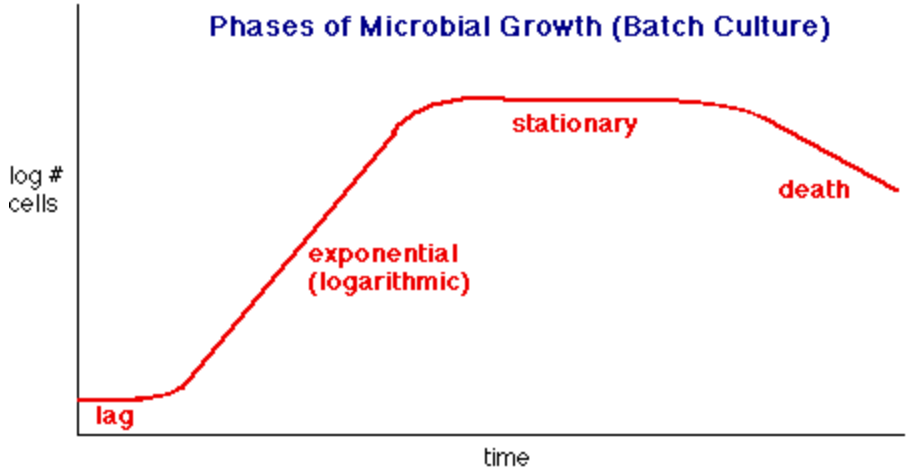

Figure 4: Showing the different stages of a growth curve.

The algal growth curve just like any other microorganism growth curve consists of a lag, log (exponential), stationary and decline phase.

The lag phase is usually the first or the initial stage of growth characterized by a lack of increase in cell numbers resulting from either a change in the environment or the introduction of medium with different metabolites than before. Although cell division seldom occurs during the lag phase the cells engage in the synthesis of key enzymes and molecules that are essential for their survival in the new environment as dictated by the vessel or contents of the medium. The length of the lag phase is dependent on a variety of factors such as the size of the inoculum, time necessary to recover from physical damage or shock encountered during the transfer, time required for synthesis of essential coenzymes or division factors, and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium (Richmond 2004; Willey, Sherwood & Woolverton 2008).

The lag phase is followed by the log or exponential phase. During the exponential phase, microorganisms begin to multiply in proportion to time. The cells divide at a constant rate that is dependent on the availability of the growth substrate as well as the incubation conditions. At this stage of growth the population is almost synchronized physiologically as well as with the culture medium in terms of chemical properties. It is for this reason that microorganisms at this

stage of growth are used in biochemical and physiological studies (Willey *et al*. 2008; Richmond 2004) as well as biotechnological production.

The exponential phase fades into the stationary phase during which the rate of cell division comes to an almost complete stop. This phase often results from the exhaustion of the available nutrients, accumulation of inhibitory metabolites or by-products and exhaustion of space. As much as this may seem undesirable, certain microorganisms have been reported to produce secondary metabolites, (secondary metabolites are defined as metabolites produced after the active stage of growth) such as antibiotics (Richmond 2004; Willey *et al*. 2008). Furthermore it's during the stationary phase that most sporulating bacteria induce genes necessary for the sporulation process.

Detrimental environmental changes like nutrient deprivation and the buildup of toxic waste lead to the decline in the number of viable cells which is the characteristic of the decline phase (Willey *et al*. 2008; Richmond 2004). One prominent feature during the decline phase is shedding. Shedding is when the microbial or algal culture starts to disintegrate.

2.6.1. Batch system

A batch system is an operational mode where a reactor is run for a given period of time without letting anything in or out. Most microalgae production systems are operated as batch reactors. In a simple batch culture system, a limited amount of the culture medium and algal inoculum is placed in a culture vessel (i.e. photobioreactor) and incubated in a favorable predetermined environment for growth. Some form of agitation, such as shaking or impeller mixing, is incorporated to necessitate an efficient gaseous exchange within the medium. The culture can be illuminated externally by either natural or artificial light sources, or internally through optical fibers placed in the culture vessels. Batch culture reactors have found a wide acceptability due to their ease of operation. For mass algal production, a portion of the culture could be retained as inoculum for the next culture batch (Pap´aˇcek 2005). The batch mode of operation is prone to all the four algal growth phases discussed previously (figure 4) (Richmond 2004).

2.6.2. Fed-Batch system

A fed-batch reactor unlike the batch reactor allows for a periodical addition of the growth medium (Pap´aˇcek 2005). This mode of operation is advantageous in that it enables the production of a higher yield of algal biomass and also through the intermittent addition of nutrients (growth medium) it delays the stationary phase which is often a result of a decrease in nutrients and it dilutes the toxic substances. A fed-batch reactor can be operated for longer than a batch reactor (Richmond 2004).

2.6.3. Continuous system

A continuous reactor as envisaged in the word is operated in a continuous mode that involves the addition of the growth medium with subsequent harvesting of the algal cells (Pap´aˇcek 2005). This mode of operation is advantageous in that it enables the production of a higher yield of algal biomass and also through the intermittent addition of nutrients (growth medium) and removal of algal cells it avoids the stationary phase which is often a result of a decrease in nutrients as well as a building up of toxic substances. A continuous reactor can be operated for years whereas batch reactors on the other hand are only operational for a short period of time (Richmond 2004). The operation of a continuous reactor relies on whether it is going to be run as a chemostat or incorporated with a turbidostat.

A bioreactor run as a chemostat involves the continuous removal of the product with the addition of fresh medium. In such reactors the volume is always kept constant. Nutrients are fed continuously at a fixed flow rate and concentration, and effluent is extracted at a fixed flow rate. A slight subtlety in the extraction part is that what is extracted is at a fixed flow rate, to keep the volume constant, but the effluent has a concentration that depends on the reaction (Liu, Li, Guo, Jiang, Li & Li 2010; Pap´aˇcek 2005; Grima, Pérez, Camacho, Sevilla & Fernández 1996; Richmond 2004).

A turbidostat on the other hand is a device that can be incorporated into a bioreactor and is used to keep the turbidity of the culture constant by manipulating the rate at which medium is fed (Pap´aˇcek 2005) and culture overgrowth removed. If the turbidity tends to increase, the feed rate is increased to dilute the turbidity back to its set point. When the turbidity tends to

fall, the feed rate is lowered so that growth can restore the turbidity to its set point (Sorgeloos, Van Outryve, Persoone & Attoir-Reynaerts 1975; Benson, Gutierrez-Wing & Rusch 2009; Rusch 2003). A turbidostat is an elaboration of the chemostat and it is particularly useful when operating under conditions which are unstable in the simple chemostat, such as high irradiance, near maximum growth rate and presence of inhibitory substrates. A turbidostat is also useful for slowly growing algae and those with a complex cell cycle (Sorgeloos *et al*. 1975; Richmond 2004). When a turbidostat is used in conjunction with a photobioreactor, under steady state conditions it is possible to determine the quantity of biomass produced per unit time.

2.7. *Spirulina* as the test alga

The choice of the test alga was made on the basis of a number of factors, the most important one being the socioeconomic importance of *Spirulina* globally. *Spirulina* has played major roles in the pharmaceutical and nutraceutical industry worldwide. However, due to the increasing demand for *Spirulina* as well as its derivatives, its production capacity ought to be increased. In order to optimize conditions in a given photobioreactor that favour an increase in algal biomass, a few factors such as morphological features, life cycle, nutritional requirements and optimal growth conditions etc. about the test organisms need to be established.

2.8. Morphology and ecology of *Spirulina*

Spirulina is a planktonic, microscopic, filamentous and multicellular photosynthetic blue green algae that derives its name from the spiral or helical nature of its filaments (Berry, Bolychevtseva, Rögner & Karapetyan 2003; Karkos, Leong, Karkos, Sivaji & Assimakopoulos 2008). Its trichrome consists of cylindrical cells of 1-12µm in diameter as represented in figure 5 (Vieira Costa, Luciane & Filho 2002; Vieira Costa, Cozza, Oliveira & Magagnin 2001; Richmond 2004). *Spirulina* can colonize environments that are unsuitable for many other organisms, forming populations in freshwater and brackish lakes and some marine environments, mainly alkaline saline lakes (Vieira Costa *et al*. 2002). The high bicarbonate content that S*pirulina* tolerates limits the growth of other algae and S*pirulina* is capable of good growth at temperature as high as 35 to 37 ^{O}C hence *Spirulina* exists almost as a uni-algal culture.

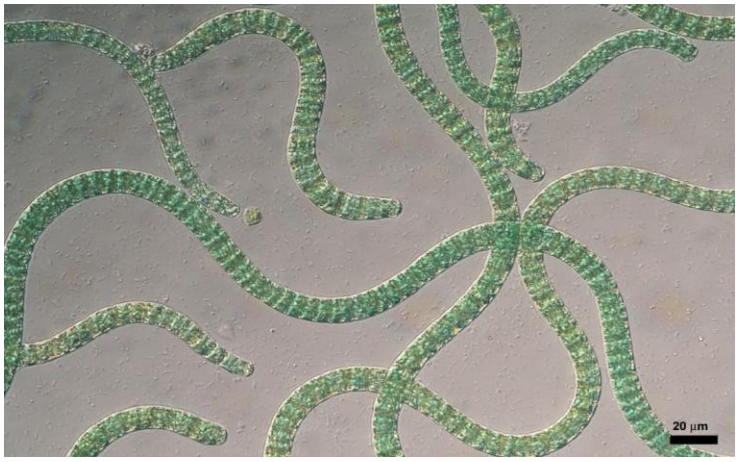

Figure 5: Microscopic image showing the spiral arrangement of Spirulina (Choonawala 2007)

2.9. Life cycle of *Spirulina*

The life cycle of *Spirulina* is relatively simple (figure 6). The trichome on maturing breaks into many fragments by forming special cells called necridia. These necridia undergo lysis to form biconcave separation disks. Thereafter, fragmentation of the trichome at necridia results in a short gliding chain of harmogonia. These specialized cells (harmogonia) detach from the parent filament and give rise to new trichome. The cells found in harmogonia lose the necridia cells and become round at the distal ends with very little thickening of the cell wall. In due course of this process the cell cytoplasm appears less granulated and the cells turn pale blue-green in colour. The cells in harmogonia increase by cell fission and the cell cytoplasm now becomes granulated. The cell assumes a bright blue green color. This process results in trichomes, which grow by length and turn into the typical helical shape. The spontaneous breakage of trichomes with formation of necridia is rarely seen in this organism (Choonawala 2007; Sánchez, Bernal-Castillo, Rozo & Rodríguez 2003; Jeamton 1997; Laorawat 1995).

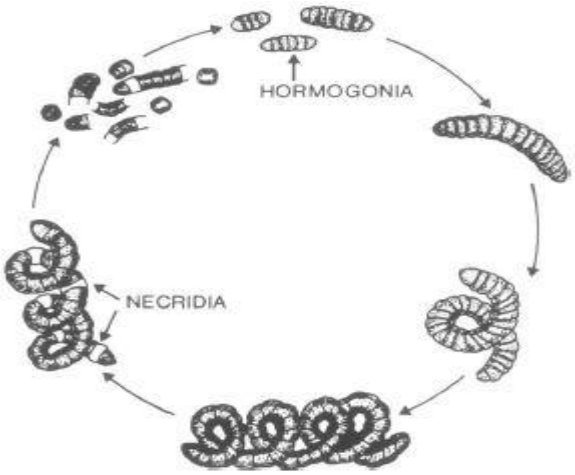

Figure 6: Showing the life cycle of Spirulina (Laorawat 1995; Choonawala 2007; Jeamton 1997; Babadzhanov, Abdusamatova, Yusupova, Faizullaeva, Mezhlumyan & Malikova 2004; Ciferri 1983).

2.10. Factors that influence the growth of *Spirulina*

In order for a culture to be cultivated successfully, various environmental and operational factors must be taken into account. These factors also affect biomass productivity as well as biomass composition. The most important factors are: nutrients, pH, temperature, light, mixing/turbulence and dissolved oxygen.

2.10.1. Nutritional and pH requirements

Carbon is an essential nutrient for *Spirulina* cultivation and can be taken from either an inorganic or organic source. Cyanobacteria have the ability to utilize CO_2, CO_3^{2-} and HCO_3^- as inorganic carbon sources. As intercellular carbon is in the form of HCO_3^- , it is converted to CO_2 by the enzyme carbonic anhydrase (CA) according to the reaction (Reuter & Müller 1993; Jaiswal, Prasanna & Kashyap 2005; Badger, Palmqvist & Yu 1994; Badger& Price 2003):

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

The CO_2 dissolved in water generates a weak acid/base buffer system, namely the bicarbonate–carbonate buffer system. The bicarbonate–carbonate buffer system is the most important buffer system generally present in natural waters (Van Vooren, Lessard, Ottoy & Vanrolleghem,

1999; Georgacakis, Sievers & Iannotti 1982). The dominance of an algal species in a system is a function of pH and temperature. As is shown in figure 7, when the pH value is about 8.5, bicarbonate species dominate but a further increase in pH above 8.5 allows the carbonate (CO_3^{2-}) species to start dominating.

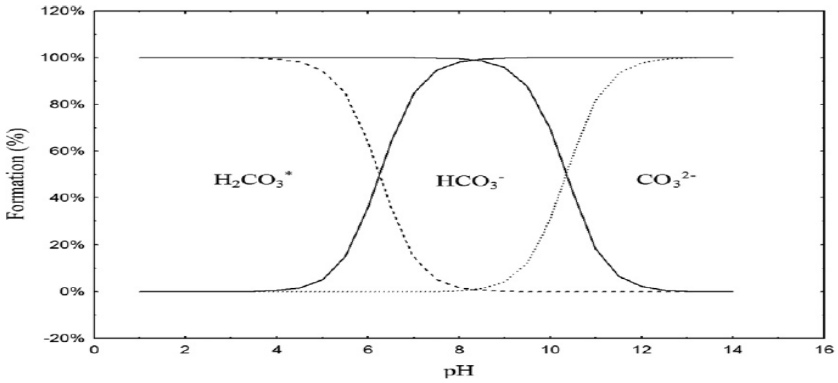

Figure 7: Relationship between pH, carbonate and bicarbonate (South & Whittick 1987; King 1978).

When the pH values are above 8.5 the cyanobacteria calcify, promoting calcium carbonate ($CaCO_3$) precipitation. In general the calcification process generates minerals and protons which are used in photosynthesis for carbon and nutrient assimilation (McConnaughey & Whelan 1997; Riding 2006; Merz 1992). The bicarbonate–carbonate buffer system provides carbon for photosynthesis through the following reactions:

$$2\ HCO_3^- \leftrightarrow CO_3^{2-} + CO_2 + H_2O$$

$$HCO_3^- \leftrightarrow CO_2 + OH^-$$

$$CO_3^{2-} + H_2O \leftrightarrow CO_2 + 2OH^-$$

Although the dissolution of CO_2 in water results in acidification due to the forming of carbonic acid, the photosynthetic process of CO_2 fixation causes a gradual rise in pH due to accumulation of OH^-. Moreover, the tendency of pH to rise is related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher (Andrade & Costa 2007). However, biomass productivity of *Spirulina* decreases in environments with pH values higher than 11 (Binaghi, Del Borghi, Lodi, Converti & Del Borghi 2003). In synthetic media for the cultivation of *Spirulina* spp. the most frequently used mediums are Schlösser and Zarrouk

medium, which provides carbon as sodium bicarbonate in the amount of 27.22 and 16.8 g/L respectively (Markou & Georgakakis 2011). In addition to inorganic carbon sources, cyanobacteria can use organic sources such as sugars, fatty acids and amino acids as a source of carbon. However, the ability to grow heterotrophically or mixotrophically on the organic substrates is species-dependent (Choonawala 2007; Markou & Georgakakis 2011).

Nitrogen is also an important nutrient for the production of micro-algal biomass. The nitrogen content of algal biomass is dependent upon the amount, the availability and the type of the nitrogen source used (Fontes, Angeles Vargas, Moreno, Guerrero & Losada 1987; Richmond 2004; Vieira Costa *et al*. 2001). Nitrogen can be assimilated in the form of NO_3^- , NO_2^- or NH_4^+ and also as N_2. Some cyanobacteria, such as *Oscilatoria*, *Anabaena* and *Spirulina*, are diazotrophic, which means that they are capable of utilizing elemental nitrogen as their sole nitrogen source by the reduction of N_2 to NH_4^+ (Benemann 1979). This process is catalyzed by the enzyme nitrogenase. The order in which cyanobacteria prefer to utilize nitrogen is NH_4^+> NO_3^- > NO_2^-. When NH_4^+ is available, cyanobacteria do not utilize other nitrogen sources until all the ammonia is utilized (Ohmori, Ohmori & Strotmann 1977). In cyanobacteria the most important route for ammonia utilization is through the glutamine synthetase enzyme system. When only nitrate is available it is reduced intracellularly by nitrate reductase to nitrite and then the nitrite is reduced by nitrite reductase to ammonia (Boussiba & Gibson 1991). The uptake of nitrate is light energy dependent, and since the reduction of nitrite consumes energy, cyanobacteria prefer to utilize already reduced nitrogen such as ammonia (Converti, Scapazzoni, Lodi & Carvalho 2006b). However, according to Vieira Costa *et al*. (2001) $NaNO_3$ is the most suitable nutrient for *Spirulina* cultivation. Nitrogen in the Schlösser or Zarrouk standard growth medium is also available in the form of sodium nitrate ($NaNO_3$).

Besides the essential nutrients mentioned above cyanobacteria also require a variety of macro- and micro-nutrients in considerable amounts. These macro-nutrients include sulphur (S), calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P). Phosphorus is perhaps one of the critical macro-nutrients needed to grow *Spirulina*. Although it is not needed in large amounts, this nutrient is an important growth limiting factor (Richmond 2004). Low phosphorus

concentration is related to low cell densities (Seale, Boraas & Warren1987). *Spirulina* utilizes the phosphorus in the form of orthophosphate (PO_4^{-3}). Micronutrients required for the cultivation of *Spirulina* include molybdenum (Mo), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cobalt (Co), boron (B), manganese (Mn) and chloride (Cl) (South & Whittick 1987).

2.10.2. Temperature requirements

Temperature is one of the major factors controlling the multiplication of *Spirulina* species. The optimum temperature for *Spirulina* growth lies in the range of 30 to 37 °C, hence S*pirulina* is a thermophilic alga (Oliveira, Monteiro, Robbs & Leite 1999). To enhance yield of biomass it is important to maintain the culture temperature as close as possible to the optimum. Temperature is an important environmental factor, which affects all metabolic activities, nutrient availability and uptake (Rafiqul Islam, Hassan, Sulebele, Orosco & Roustaian 2003). At high temperatures (30 to 37 °C), *Spirulina* can grow healthily and the productivity can be improved. At lower temperatures, *Spirulina* does not grow very well and result in lower biomass production.

2.10.3. Light requirements

As with all plants, algae photosynthesize, i.e. they convert carbon dioxide into organic compounds, especially sugars, using light as a source of energy. Thus light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture. The growth rate of microalgae increases in proportion with increasing light intensity until saturation levels (figure 8) (Oncel & Akpolat 2006). Cell division, an aspect of cell growth, is also known to be influenced by light intensity. Sorokin & Krauss (1965) reported that an increase in light intensity first favors cell division then, after the optimal light intensity is attained, a further increase in light intensity inhibits the cell division process. Light may be supplied by natural sunlight or by commercially available photosynthetic light sources (fluorescent tubes and LEDs) (Ewings 2005). Although the wavelength range of solar radiation is very broad, only radiation between 400 and 700 nm can be used by microalgae. This part of the solar spectrum is called “Photosynthetic Active Radiation” (PAR) (Acien Ferna´ndez, Garcia

Camacho, Sanchez Perez, Fernandez Sevilla & Molina Grima 1998; Janssen 2002). Higher light intensity (e.g. direct sunlight or small container close to artificial light) may result in the damage of the photosynthetic apparatus of the Photosystem II and this process is referred to as photo-inhibition. Also, overheating due to both natural and artificial illumination can take place especially in photobioreactors. Fluorescent tubes emitting either the blue or the red light spectrum are preferred as these are the most active portions of the light spectrum for photosynthesis (Ewings 2005).

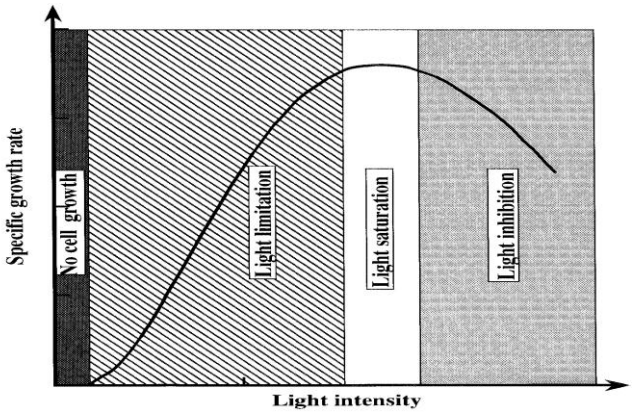

Figure 8: A general relationship between light intensity and specific growth rate (Ogbonna & Tanaka 2000). This is usually culture specific.

2.10.4. Mixing/Turbulence

Mixing and turbulence are critical for algal biomass production (Grobbelaar 2008; Grobbelaar 1991; Grobbelaar 2000; Qiang *et al*. 1998). To achieve this some form of agitation must be provided so as to enable an even distribution of the algal culture within the growth medium. Mixing mitigates the sedimentation of the algae, thus ensuring that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (e.g. in outdoor cultures) and this also improves gas exchange between the culture medium and the air thereby presenting an added advantage.

2.10.5. Dissolved oxygen

Photosynthetic production of algae is always accompanied by evolution of oxygen and consumption of carbon dioxide. Oxygen levels above air saturation (0.2247 mol O_2 m^{-3} at 20 °C)

can inhibit photosynthesis in many algal species, even if carbon dioxide concentration is maintained at elevated levels (Camacho Rubio, Acie´n Fernandez, SancheZ Pe´ rez., Garcıa Camacho & Molina Grima 1998; Ugwu, Aoyagi & Uchiyama 2007). A clear decrease of *Spirulina* biomass formation is found when dissolved oxygen is greater than 25 mg l^{-1} (315 %) (Jiménez, Cossío & Niell 2003b). In addition, elevated levels of oxygen combined with high levels of irradiance, can lead to severe photo-oxidation (Torzillo, Pushparaj, Masojidek & Vonshak 2003; Ma & Gao 2009). Therefore, an important aspect of design and scale-up of tubular photobioreactors is establishing combinations of tube length, flow rate, and irradiance levels that do not allow oxygen build-up to inhibitory levels (Camacho Rubio *et al*. 1998; Cheng, Zhang, Chen & Gao 2006b).

2.11. Ways of measuring cell growth

Gravimetric estimation of algal dry weight is one of the most direct ways to determine biomass production. This parameter only characterizes the general physico-chemical state of the given sample (which may include extraneous material other than biomass) and cannot always be applied as the sole method for biomass estimation because it does not differentiate between the actual algal biomass and suspended non-biological solids. A specific volume of the culture is filtered through a dry pre-weighed filter. The filter is re-weighed after drying on a precision balance until constant mass is achieved (Rafigul, Jala & Alam 2005; Pandey & Tiwari 2010; Pandey, Tiwari & Mishra 2010b; Pandey, Pathak & Tiwari 2010a; Choonawala 2007; Algal Biomass Organisation 2010; Uslu, Isik, Koc & Goksan 2011; Tolga, Ayßeg & Ülknur 2006; Richmond 2004).

Another way of measuring algal cellular growth is through the turbidity. Measurement of the turbidity with a spectrophotometer or with a turbidity meter is the most general method for estimating the algal concentration of suspended solids in the culture (Algal Biomass Organisation 2010). The amount of light absorbed by a suspension of cells can be related directly to cell mass or cell number (Griffiths, Garcin, Van Hille & Harrison 2011). Spectrophotometric analysis is the measurement of the optical density at a given wavelength.

This method is based on the application of the photometric law which states that each elementary layer of a dispersed system scatters the same relative portion of the passing monochromatic light, the magnitude of the scatter being proportional to the dispersion concentration. A wavelength of 680 nm is recommended (Choonawala 2007; Griffiths *et al*. 2011; Uslu *et al*. 2011; Çelekli & Yavuzatmaca 2009; Çelekli, Yavuzatmaca & Bozkurt 2009; Tolga *et al*. 2006).

Although cumbersome, microscopic examination could also be used for algal quantification. However the added advantage to this method is that one can actually confirm the identity of the algal species that is growing. This method makes use of a counting chamber known as a haemocytometer. Reproducibility of the counts is however the main problem with this procedure so attention must be given to sampling, dilution of the medium and filling of the chamber.

Other complex equipment such as the flow cytometer or cell Coulter counter could also be used to quantify the algae in a given sample. However the main drawback with such methods is that they can be time-intensive if done manually and can also be limited by cell size and complicated by large size distribution or by filamentous or a multicellular or colonial morphology (Algal Biomass Organisation 2010; Choonawala 2007; Uslu *et al*. 2009).

2.12. Commercial production of *Spirulina*

Spirulina has been commercially cultivated for the production of food supplement in human diets and animal feed (Thammathorn 2001; Rodrigues, Ferreira, Converti, Sato & Carvalho 2010). This organism at one point in history (some 400 years ago) served as a major source of food in Mexico during the Aztec civilization. To date *Spirulina* is still regarded as a source of food ("dihe") by the Kanembu tribe in the Lake Chad area of the Republic of Chad (Belay 2002). *Spirulina* is considered an excellent food source because of properties such as the lack of toxicity, and its corrective properties against viral attacks, anaemia, tumour growth and

malnutrition. For these reasons, there is an increasing interest in *Spirulina* cultivation, and its large-scale production (Table 2) is under consideration worldwide (Thammathorn 2001).

Table 2: Commercial production of *Spirulina*(Thammathorn 2001; Rodrigues *et al*. 2010).

Company	Country	Total pond area (m2)	Productivity (tonnes per Year)
Ballarpur Industries Ltd.	India	54,000	25
Cyanotech Corporation	USA	100,000	250
Earthrise Farms	USA	150,000	360
Myanma Microalga Biotechnology	Myanmar	130,000	32
Siam Algae Co., Ltd.	Thailand	44,000	125
Wuhan Microalgal Biotechnology Company	China	20.000	25
Nan Pao Resin Chemical Co.,Ltd.	Taiwan	50,000	70

2.13. Nutritional and chemical composition of *Spirulina*

Spirulina, like other microalgae, is capable of producing valuable metabolites, such as pigments, proteins and vitamins for feed additives, and for pharmaceutical and nutraceutical purposes (Tolga *et al*. 2006). These organisms are very important since they seem to be at the core of the food chain within various ecosystems (Dernekbasi, Una, Karayucel & Aral 2010). A cross examination of *Spirulina* powder (Figure 9) showed that this microalga is composed of 65%

protein, 15% carbohydrate, 6.5% fats, 7% minerals and 3-6% moisture making it a low-fat, low calorie and cholesterol-free source of protein.

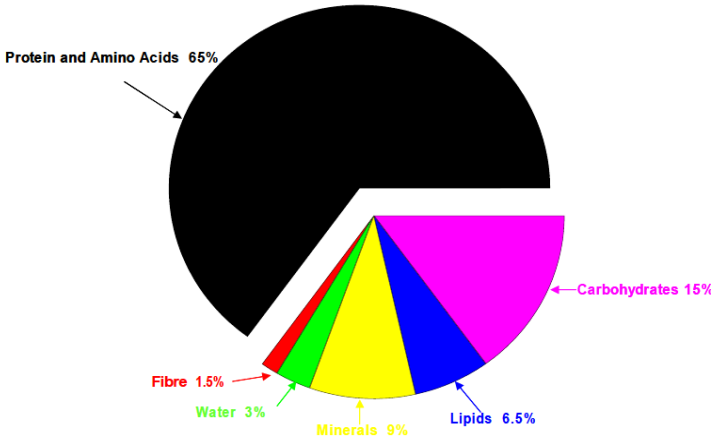

Figure 9: Chemical and nutritional composition of Spirulina (Choonawala 2007; Dernekbasi et al. 2010; Paredes-Carbajal, Torres-Durán, Díaz-Zagoya, Mascher & Juárez-oropeza 1997).

2.13.1. Protein and amino acid content

Spirulina is a rich source of protein (between 50% and 70% protein by dry weight) (Ahsan, Habib & Parvin 2008; Pelizer, Danesi, Rangel, Sassano, Carvalho, Sato & Moraes 2003; Morais, Radmann, Andrade, Teixeira, Brusch & Costa 2009; Jiménez, Cossío, Labella & XAVIER NIELL 2003a) in comparison to other sources such as meat and fish that contain 15-20% protein, 35% in soy, 35% in evaporated milk, 25% in peanuts, 12% in eggs and in grains 8-14% (Babadzhanov *et al*. 2004; Paredes-Carbajal *et al*. 1997). In addition *Spirulina* protein is 90% digestible and contains enzymes, which assist the digestion process (Dernekbasi *et al*. 2010; Nandeesha, Gangadhara, Manissery & Venkataraman 2001). Furthermore, the protein is of high quality (Becker & Venkataraman 1984); the balance of amino acids compares well with the "ideal" protein standards established by the protein Advisory Group of the Food and Agriculture Organization of the United Nation (Dernekbasi *et al*. 2010; Sassano, Gioielli, Ferreira, Rodrigues, Sato, Converti & Carvalho 2010; Pelizer et al. 2003; Danesi, De, Rangel-Yagui, De Carvalho & Sato 2002). The crude protein from *Spirulina* contains methionine, tryptophan and other

essential amino acids (table 3) at concentrations similar to, if not higher than those present in milk (Ahsan *et al*. 2008).

Table 3: Amino acid composition of *Spirulina*(Babadzhanov *et al*. 2004).

Amino acids	%	Amino acids	%
Asp	0.9	Met	0.8
Thr	0.5	Yle	1.3
Ser	0.6	Leu	0.8
Glu	1.0	Tyr	3.3
Pro	0.3	Phe	2.5
Gly	0.6	His	4.7
Ala	1.0	Lyz	1.9
Val	1.3	Arg	2.1

2.13.2. Unsaturated fatty acids and lipids

The essential lipids (unsaturated fatty acids) in *Spirulina* are about 1.3–15% of total lipid content (table 4), and mainly constitute of γ-linolenic acid (30–35% of total lipid).

Table 4: Properties of *Spirulina* lipids(Babadzhanov *et al*. 2004)

Fatty acids	mg/kg biomass	mass % of fatty acids
12:0	437	0.4
14:0	874	0.8
16:0	49031	44.9
16:1	2512	2.3
17:0	1310	1.2
18:0	2402	2.2
18:1	11029	10.1
18:2	12121	11.1
18:3	18672	17.1
others	10811	9.9

Important fatty acids like linoleic acid and linolenic acid (table 4) are also present (Piorreck, Baasch & Pohl 1984; Danesi *et al*. 2002): up to 1.0 g/100 g of dry biomass of *Spirulina*. The predominant fatty acids are palmitic acid (44.6–54.1%), oleic acid (1–15.5%), linoleic acid (10.8–30.7%) and γ-linolenic acid around 8.0–31.7% (Ahsan *et al*. 2008; Piorreck *et al*. 1984; Pham

Quoc& Dubacq 1997; Cohen, Vonshak & Richmond 1987; Cohen & Vonshak 1991; Sajilata *et al*, 2008).

2.13.3. Carbohydrates

In general, carbohydrates constitute 15-25% of the dry weight of *Spirulina* (Danesi *et al*. 2002). Simple carbohydrates such as glucose, fructose and sucrose are present in small quantities and polyols such as glycerol, mannitol and sorbitol also do occur. Polysaccharides such as rhamnose and glycogen are easily absorbed by human cells with minimal intervention by insulin. Hence, *Spirulina* sugars provide speedy energy, without taxing the pancreas or precipitating hypoglycemia. From a nutritional point of view, the only carbohydrate of interest that occurs in sufficient quantities is mesoinositol phosphate. Mesoinositol phosphate is an excellent source of organic phosphorus and inositol (Mendiola, García-Martínez, Rupérez, Martín-Álvarez, Reglero, Cifuentes, Barbas, Ibañez & Señoráns 2008). This inositol is about eight times that of beef and several hundred times that of vegetables.

2.13.4. Vitamins

The β-carotene, B-group vitamins, vitamin E, iron, potassium and chlorophyll available in *Spirulina* can promote the metabolism of carbohydrates, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa (Mazo, Gmoshinskiĭ & Zilova 2004; Danesi *et al*. 2002). *Spirulina* contains large amounts of natural β-carotene and this β-carotene is converted into vitamin A (Kulshreshtha, Zacharia, Jarouliya, bhadauriya, prasad & Bisen 2008). According to the findings of the National Cancer Institute, United States of America, an intake of 6.0 mg β-carotene daily may be effective in minimizing the risk of cancer (Mendiola *et al*. 2008). If anybody takes 4.0 g *Spirulina* daily, that is sufficient to get 6 mg β-carotene. At the same time, sufficient amounts of B-group vitamins, iron and calcium will be obtained. However, these nutrients obtained from 4.0 g of *Spirulina* are equivalent to or more than those obtained by eating more than 100 g of terrestrial brightly coloured vegetables.

2.13.5. Minerals

About 9% of the total dry weight is composed of essential minerals, potassium, magnesium, calcium, zinc and iron (Duan, Ma, Li & Sun 2001; Cheng, Hong, Li, Fan & Cai 2006a; Zaretskaia, Gmoshinskiĭ, Mazo, Zorin & Aleshko-Ozhevskiĭ 2004). The waters *Spirulina* favours are saturated with minerals deposited from ancient soils and mountains and because *Spirulina* thrives in such alkaline waters, it incorporates and synthesizes many minerals and derivative compounds into its cell structure.

2.13.6. Photosynthetic pigments

Photosynthesis is the most abundant energy-storing and life supporting process on earth. Cyanobacteria are a valuable ubiquitous component of the aquatic system that contributes significantly to the total photosynthetic products. *Spirulina* among cyanobacteria is attracting commercial conglomerates as a source of various nutraceuticals, biomass and pigments (Madhyastha & Vatsala 2007). Among various pigments with growing commercial importance due to their high end application and easy extraction procedures are chlorophyll a, xanthophyll, β-carotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin (Ahsan *et al*. 2008; Cheng *et al*. 2006a; Leema, Kirubagaran, Vinithkumar, Dheenan & Karthikayulu 2010).

2.14. Literature summary

In this study a novel tubular photobioreactor with the ability to expose the cultured algae to light/ dark cycles was considered for the cultivation of algae at a small pilot plant scale both indoors (laboratory) and outdoors. This type of bioreactor was chosen based on the reasons stated above in section 2.2 and 2.5. The alga *Spirulina* was chosen as the test organism for growth in the novel tubular photobioreactor. In brief, *Spirulina* was chosen due to the fact that it could easily be cultivated, tolerates extreme conditions in comparison to most microalgae, and for its socioeconomic impact on society. The novel tubular photobioreactor was optimized so as to favour high *Spirulina* biomass production.

CHAPTER 3

3. RESEARCH AND METHODOLOGY

3.1. Introduction

The aim of this study was to develop a novel tubular photobioreactor and use it to optimize the production of algal biomass. In order to achieve this aim a tubular photobioreactor had to be constructed. After the construction of the photobioreactor, a test algal species had to be selected and studied in the laboratory before growing it in the photobioreactor. This was followed by the optimization of the novel tubular photobioreactor for optimal algal biomass production.

3.2. The construction of the tubular photobioreactor

The photobioreactor was constructed using steel bars, a polyvinyl chloride (PVC) tube, a 220 volt pump, lights (fluorescent and LED lights), a steel box with wheels, reservoirs with plastic water connections and electrical connections. All these items are readily available within South Africa. The photobioreactor was constructed in such a way that its top part was exposed to light while the bottom part was kept in the dark.

The top part was constructed by arranging four 1.2m steel bars in a pyramidal-like structure that was supported by screws with cross bars at the top and the middle. Smooth edged metal hooks were then attached to each of the metal bars at the same interval allowing a maximum of 5mm between each other. The pyramidal structure with hooks was then painted white and set aside to dry.

The bottom part was constructed in the form of a box (width of 1m by 1m and a height of 0.5m) using steel plates with the exception of the bottom were wood (2cm thick) was used and supported on an angle iron frame. The top was then over-laid with fibre glass in order to prevent the accumulation of water. The box was then fitted with wheels and painted white on the exterior and black in the interior.

After the paint had dried the top part was fitted onto the bottom part and reinforced with screws at the bottom. Following this, lights were mounted on the four sides of the pyramidal – like structure and supported by the middle cross bars and the top part of the box. Then a 150m transparent PVC tube was placed on the hooks all around the pyramidal-like structure of the photobioreactor and connected to the bottom part of the photobioreactor through a plastic plumbing connection.

A 220 volt pump was fitted inside the box by screwing it to the bottom wooden part. The pump by means of a plastic plumbing connector was connected to the PVC tube on the top part of the photobioreactor. Reservoirs were incorporated in the bottom part of the photobioreactor based on the nature of the experiment. A reservoir was a 20 litre bucket and was connected to the pump through tubing and plastic plumbing connections. Electrical connections were used to run the pump and lights.

The photobioreactor was maintained and cleaned with a sponge ball and chlorinated water after each experiment. Following the chlorinated water treatment the system was purged with copious amounts of water.

3.3. Organism and culture conditions

The cyanobacterium *Spirulina platensis* was used in this study. This strain of *Spirulina* which was previously maintained in Schlösser growth media was obtained from the University of North West, South Africa. The culture was transferred from Schlösser agar slant into a conical flask containing Schlösser liquid growth media (as described in Appendix A). The culture was incubated in a dark room where it was continuously stirred and illuminated with 36 W Osram fluorescent lamps (Osram, Germany). The photon flux density (PFD) on the surface of the flask was set at 120 µmol m^{-2} s^{-1} by manipulating the distance of the lamps. The light intensity was measured by a quantum meter (BQM 1-800-248-8873, Apogee instruments Inc). This culture was used as a stock culture for conducting the research.

3.4. Evaluation of *Spirulina platensis*'s growth dynamics

A 2000 ml flask was used in which 1800 ml of the Schlösser growth medium was added together with 200 ml of the *Spirulina platensis* culture. The culture was continuously stirred and illuminated at 120 µmol m^{-2} s^{-1} light intensity. Samples were taken on a daily basis for the measurement of pH (HANNA HB 98128), temperature (HANNA HB 98128) and for determining biomass concentration.

Biomass concentration of the culture was determined by dry weight and optical density measurement. For dry weight, a sample containing 25 ml of the *Spirulina* suspension was filtered through a 0.45 µm pore size Whatman GF/C filter paper which was preweighed on a 0.01 mg precision balance (SHIMADZU AUW220D, Japan). The filter paper was dried at 80 ^{0}C in an oven for 12 to 18 hrs. After drying the filter paper was weighed and the difference between the initial and final weight were taken as the dry weight of *Spirulina* biomass. Dry weight was expressed in terms of grams per liter (g/l). Samples were taken and analyzed in duplicate.

Calculation

$$\frac{\text{(weight of filter paper (g) + algae (g)) - (weight of filter alone (g)) x lOOO}}{\text{Volume of algal in ml}}$$

= algal dry weight in $mg.l^{-1}$ or $g.l^{-1}$

Optical density was determined spectrophotometrically at 680 nm in a UV/Visible spectrophotometer (Nanocolor UV/VIS, Germany). A 1 cm light path cuvette was used.

3.5. The operation of the novel tubular photobioreactor

The novel tubular photobioreactor was operated both indoors (using artificial lights) as well as outdoors (using artificial and natural sunlight (1500 µmol m^{-2} s^{-1})). The indoors operation of the photobioreactor was divided into two sections, namely low light intensity (30, 60 and 120 µmol m^{-2} s^{-1}) and high light intensity section (600 µmol m^{-2} s^{-1}).

3.5.1. Low light intensity section

3.5.1.1. The operation of the novel tubular photobioreactor at different low artificial light intensities

The reactor was started with a 10 % inoculum of *Spirulina* and 72 litres of the Schlösser growth medium to make a total volume of 80 litres. The algal culture was illuminated with florescent lights at a light intensity of 120µmol m^{-2} s^{-1}. The culture was continuously exposed to a light and a dark phase by a diaphragm pump at a light/ dark ratio of 1:1.4. Temperature was maintained between 33 and 37 ^{0}C by heating the room with external heaters. Samples were taken on a daily basis for pH and temperature measurement as well as for the analysis of biomass and optical cell density according to the methods mentioned in section 3.4 of the methodology. The experiment was conducted for a period of 25 days. The same procedure was followed when the light intensity was reduced to 60 and 30µmol m^{-2} s^{-1} respectively.

3.5.1.2. The operation of the photobioreactor at varying light/dark cycles

A 10 % *Spirulina* inoculum was used to inoculate a novel tubular photobioreactor with a total volume of 70 litres. The algal culture was continuously exposed to a light (artificial illumination) and a dark phase at a ratio of 1:1. Temperature was kept constant by heating the room/laboratory with external heaters. Samples were taken on a daily basis for temperature and pH measurement as well as the analysis of biomass and optical density according to the method in section 3.4 of the methodology. The experiment was conducted until a stationary phase of the growth curve was reached. The same procedure was repeated when the ratio of light to dark phase/cycle was increased to 1:1.3 and 1:1.4. The photobioreactor was also operated using both artificial and natural light in which the procedure explained above was used at ratios of 1:1 and 1:1.4.

3.5.2. High light intensity section

3.5.2.1. The operation of the photobioreactor under continuous illumination

The novel tubular photobioreactor was started with a 10 % *Spirulina* inoculum. The total volume of the photobioreactor was 25 litres. 23 litres of the photobioreactor's total volume was continuously illuminated by a combination of high intensity LED's and fluorescent lights (in the light phase). The light intensity of LED's and fluorescent lights in the light phase of the photobioreactor measured at different levels/parts (top, middle and bottom at different sides) of the photobioreactor and the average light intensity was found to be 600 µmol m^{-2} s^{-1} .The remaining 2 litres of the photobioreactor's total volume were kept in the reservoir in the dark phase for degassing and the culture was also continuously illuminated by fluorescent lights (120 µmol m^{-2} s^{-1}) placed on top of the reservoir. This meant that the total volume of the reactor was continuously illuminated. The culture was also continuously circulated from the light phase into the dark phase (but the dark phase was also illuminated as explained above) by a diaphragm pump. Samples were taken on a daily basis for pH and temperature measurement as well as for the analysis of biomass and optical density according to the method in section 3.4 of the methodology.

3.5.2.2. The operation of the photobioreactor at varying light/dark cycles

A novel tubular photobioreactor with a total volume of 46 litres was started with a 10 % *Spirulina* inoculum. The algal culture was continuously exposed by a diaphragm pump to a light phase (with a light intensity of 600 µmol m^{-2} s^{-1} as explained in section 3.5.2.1 of the methodology) and a dark phase at a light/ dark ratio of 1:1. The photobioreactor was operated in a batch mode for a period of 14 days in this light/ dark ratio with samples taken on a daily basis for the measurement of pH, temperature, optical density and biomass. The same procedure was repeated when the ratio of light to dark cycle was 1:1.5, 1:2, 1:0.5, 1:0.25 and 1:0.125. The best light to dark ratio that produced the highest biomass in the shortest number

of days when compared to other ratios (as explained in 3.3.2.1) was observed. This ratio (the best ratio) was then used to operate the novel tubular photobioreactor in a continuous mode.

3.6. The operation of the photobioreactor as a continuous system using LED lights.

The novel tubular photobioreactor was started with an initial volume of 26.1 litres of the Schlösser growth medium and 2.9 litre of *S. platensis* inoculum. The culture was illuminated with fluorescent and high intensity LED lights at a light intensity of 600 µmol m^{-2} s^{-1}. Temperature was kept at its optimum by heating the room (laboratory) with external heaters. Samples were taken on a daily basis for pH and temperature measurements as well as for the analysis for biomass and cell density according to the method mentioned in section 3.4 of the methodology. When the *S. platensis* cell density reached a predetermined level (1.7 optical density), the photobioreactor was transformed from a batch mode of operation into a continuous mode through the incorporation of a turbidostat. The turbidostat was used to maintain the algal cell density at a constant dry weight of 1.5 g/l. The turbidostat consisted of a photocell sensor, light source, a pump and a control system box. The photocell and the light source were installed opposite one another in a tube (20 mm PVC tubing used to construct the photobioreactor) connecting the diaphragm pump in the dark phase to the light phase of the photobioreactor. When the cell density was higher than the set point (cells too dense hence blocking the light which results in the light sensor not sensing light), the control point would trigger the pump to start adding fresh growth medium and at the same time a volume of the culture (cultured alga) that was equal to the volume of the added fresh growth medium would be removed until the cell density was diluted back to its set point.

CHAPTER 4

4. RESULTS

4.1. Introduction

In this study a novel tubular photobioreactor was constructed. This was followed by the determination of the optimal growth conditions of *Spirulina Platensis* at laboratory scale. The photobioreactor was operated at a small pilot plant scale both indoors (laboratory) and outdoors. The effect of the dark/light cycles on *Spirulina Platensis* biomass production was evaluated using artificial (commercial lights in the laboratory) and natural (sunlight outside the laboratory) light sources. The photobioreactor was operated as a batch and continuous system. Dry cell mass as well as turbidity was used to monitor cell growth (in the novel tubular photobioreactor) with respect to biomass production in both systems. These parameters were followed continuously and correlated to the different dark/ light cycle regimes.

4.2. The construction of the photobioreactor

The photobioreactor consisted of two physical phases namely the light (the top part of the reactor) and dark phase (bottom part of the reactor) as shown in figure 10.

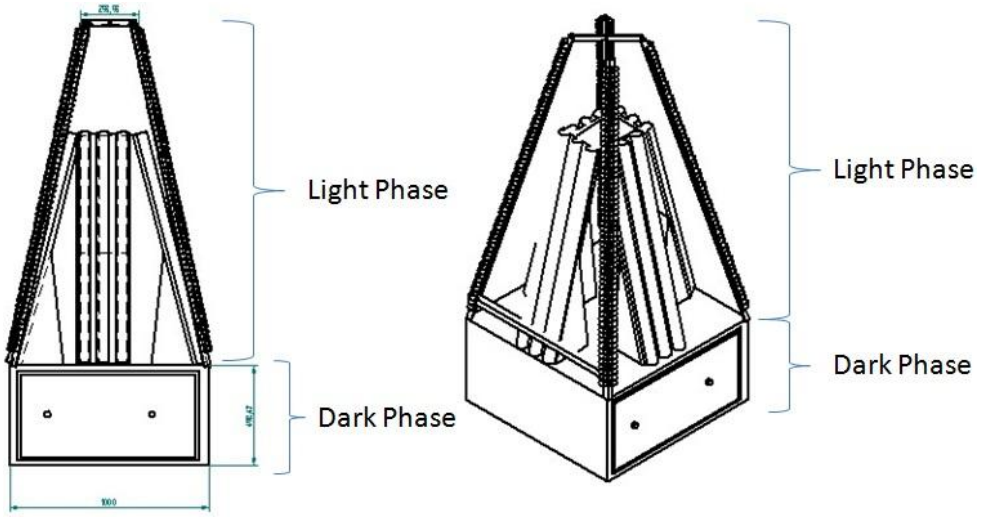

Figure 10: Showing the light phase and the dark phase of the photobioreactor.

The light phase of the reactor was constructed in the form of a pyramid-like structure as shown in figure 11 below. This part was easily illuminated by either natural sunlight or with artificial lights. The tubing used was transparent and allowed for the transmission of photosynthetic active radiation (PAR) through the material.

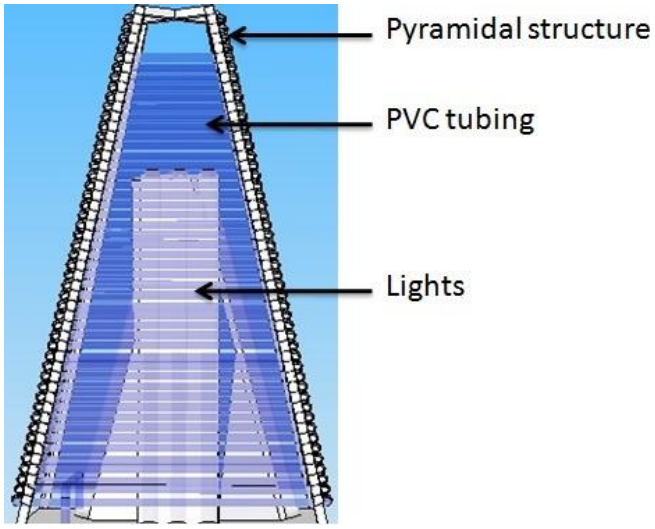

Figure 11: Showing the light phase of the bioreactor.

The dark phase of the photobioreactor was encapsulated in an opaque box to which the light phase was attached (Figure12).

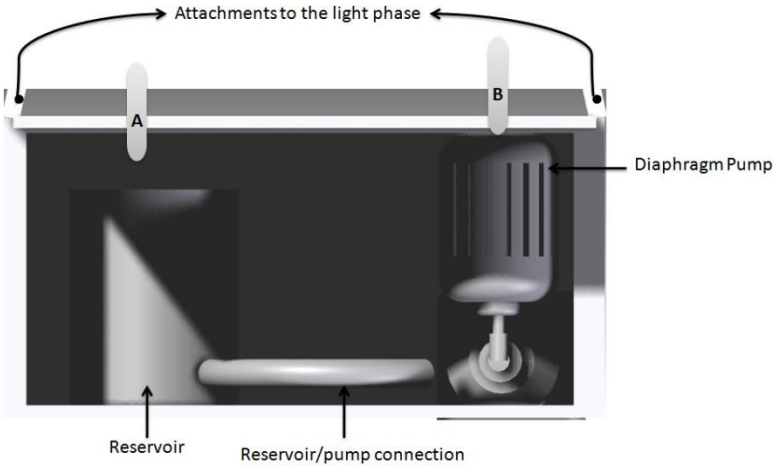

Figure 12: Showing the content of the dark phase of the bioreactor. The letter A denotes the inlet from the light phase into the dark phase whereas letter B denotes the outlet from the dark phase into the light phase through the diaphragm pump.

The diaphragm pump was used to circulate the reactor's contents thorough an outlet as shown in figure 12. This novel tubular photobioreactor with both light and dark phases (as shown in figure 13) has been patented (Appendix B and C).

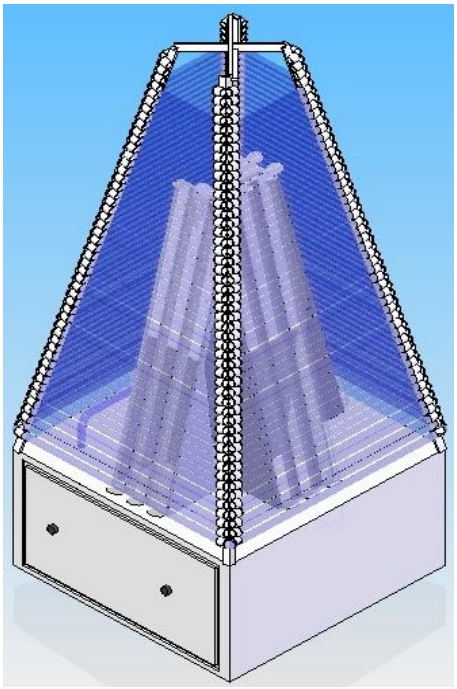

Figure 13: Novel tubular photobioreactor.

4.3. Evaluation of growth conditions of the inoculum

The test organism chosen for this project was *Spirulina platensis* for reasons stated in section 2.7 of the introduction. This organism had previously been maintained on Schlösser agar media. Before the alga could be cultivated in the novel tubular photobioreactor, it was essential to establish an inoculum. The algal inoculum was established in the laboratory using Schlösser liquid growth medium, the same medium that would be used during the operation of the novel tubular photobioreactor. A growth curve of the alga in this medium was determined for a period of 18 days with subsequent measurement of the pH and temperature.

The results seemed to indicate that the Schlösser growth media was suitable for the growth of *Spirulina platensis.* This alga showed an exponential increase in growth as indicated by both the OD_{680nm} and biomass readings although most of the growth curve was mainly made up of the log phase and an early stationary phase (figure 14).

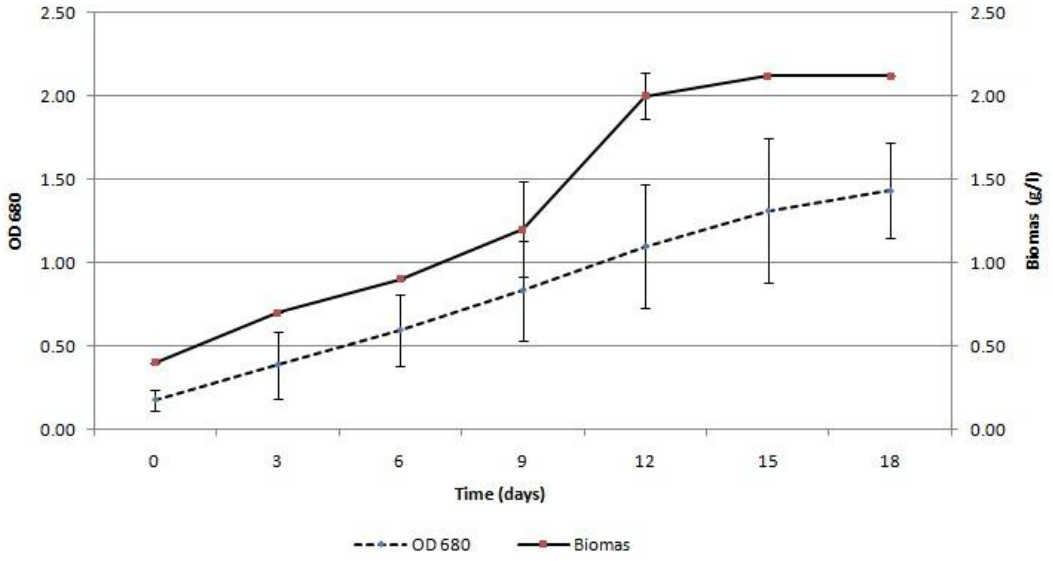

Figure 14: Showing a growth curve of Spirulina platensis in Schlösser liquid growth medium grown for a period of 18 days. The error bars indicate duplicate independent experiments.

Although the temperature remained between 25 and 30 ^{o}C, a gradual increase in the pH was observed. The initial pH was 9.3 but after 18 days there had been an increase in the pH by 0.7 units (figure 15).

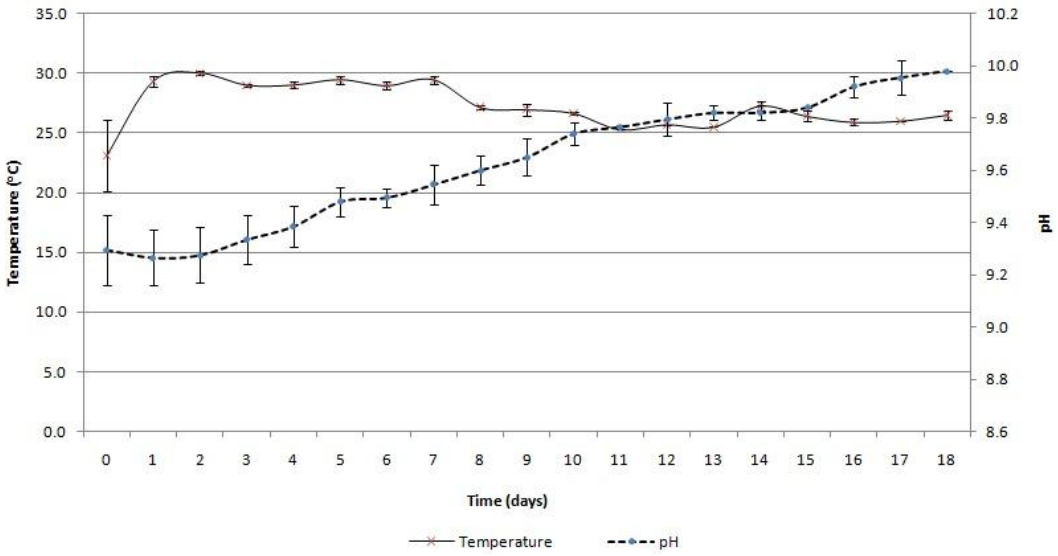

Figure 15: Showing the pH and Temperature values during the 18 days of growth of Spirulina platensis in Schlösser liquid growth medium.

4.4. The effect of light intensity on the growth of *Spirulina platensis* in the novel tubular photobioreactor

Following the establishment of the inoculum in the laboratory the operation of the novel tubular photobioreactor commenced. The first task during the batch operation of the photobioreactor for algal biomass formation was to examine the effect light might have had on the growth of *Spirulina platensis.* During the preparation of the inoculum this alga was exposed to a light intensity of 120 µmol m^{-2} s^{-1} since this was the maximum light intensity that the fluorescent lamps used could emit. The same light intensity (120 µmol m^{-2} s^{-1}) was emitted by the fluorescent lights used in the light phase of the photobioreactor. In order to optimize the photobioreactor for maximal algal growth it was important to determine the effect that light intensity had on the biomass production of this alga. This was done by subjecting the alga for a duration of 25 days to its maximum light intensity which was then reduced to 60 and 30 µmol m^{-2} s^{-1} respectively for the same duration. Results showed that there was a directly proportional relationship between light intensity and biomass formation (figure 16). Maximum algal dry biomass formation was achieved when the reactor was operated at its maximum light intensity (120 µmol m^{-2} s^{-1}).

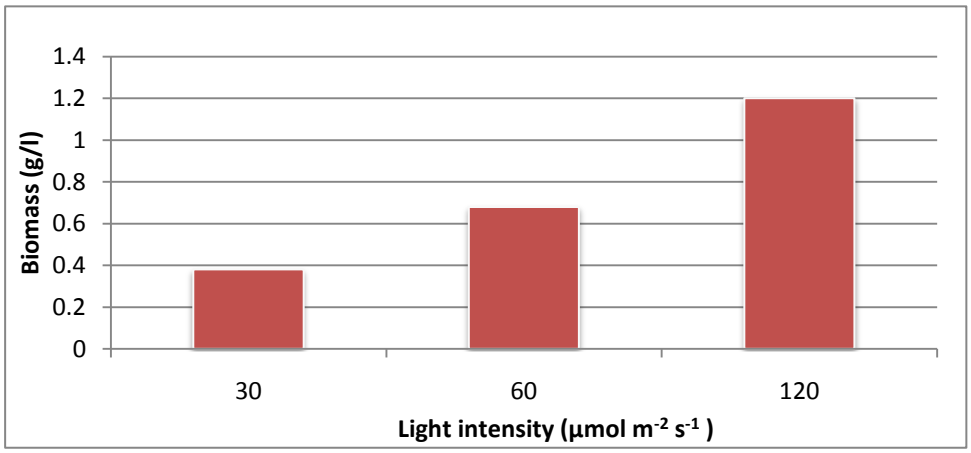

Figure 16: Showing algal dry biomass production when grown in the photobioreactor at different light intensities for a period of 25 days.

However, when the light intensity was reduced by a magnitude of half (60 µmol m^{-2} s^{-1}), there was almost a similar reduction in the magnitude of dry biomass formation. This was also observed when the light intensity was further reduced to 30 µmol m^{-2} s^{-1}. The specific growth

rate of this alga under these three light intensity treatment regimens also increased with increasing light intensity. The specific growth rate was at its lowest (0.204) when algal cells were exposed to a light intensity of 30 µmol m^{-2} s^{-1} in comparison to when the light intensity was 60 and 120 µmol m^{-2} s^{-1}, i.e. 0.516 and 1.207 respectively.

When LED lights known to emit higher light intensity (600µmol m^{-2} s^{-1}) than fluorescent lights (120 µmol m^{-2} s^{-1}) were used, an increase in dry biomass was not directly proportional to an increase in light intensity as previously observed. It was also found that the cells illuminated with LED lights had a much higher specific growth rate in comparison to when they were illuminated by fluorescent lights (figure 17). As a result these algal cells reached their stationary phase within a shorter period of time and when left longer than 14 days a drastic colour change from green to yellow was observed.

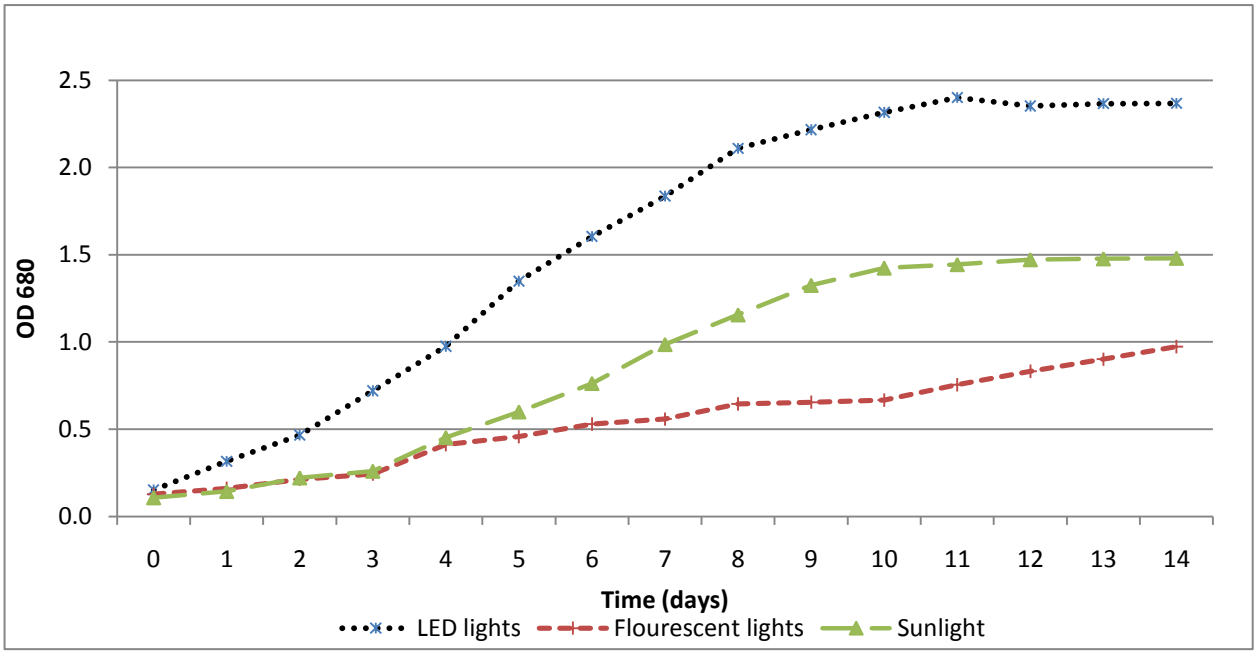

Figure 17: Showing a comparison between the LED, fluorescent and sunlight results obtained in 14 days.

When the photobioreactor was run with a combination of sunlight during the day and fluorescent tubes during the night, no further increase in dry biomass formation by the alga was observed when compared to that achieved when the photobioreactor was illuminated by LED lights alone (figure 17). The light intensity emitted by the sun (1500 µmol m^{-2} s^{-1}) was much

higher than that emitted by the LED lights and was dependent on the conditions of the sky (cloud cover).

On the other hand, important parameters such as temperature and pH were monitored during the investigation of the effect that light intensity might have had on biomass formation. When the photobioreactor was operated in the laboratory the temperature of the algal culture was maintained at 36 ^{o}C ± 2 ^{o}C. But when the photobioreactor was exposed to natural environmental conditions the temperature of the algal culture fluctuated between 40 ^{o}C (during the day) and 25 ^{o}C (during the night). It was also observed that there was a relationship between change in pH (Δ pH) and light intensity (figure 18).

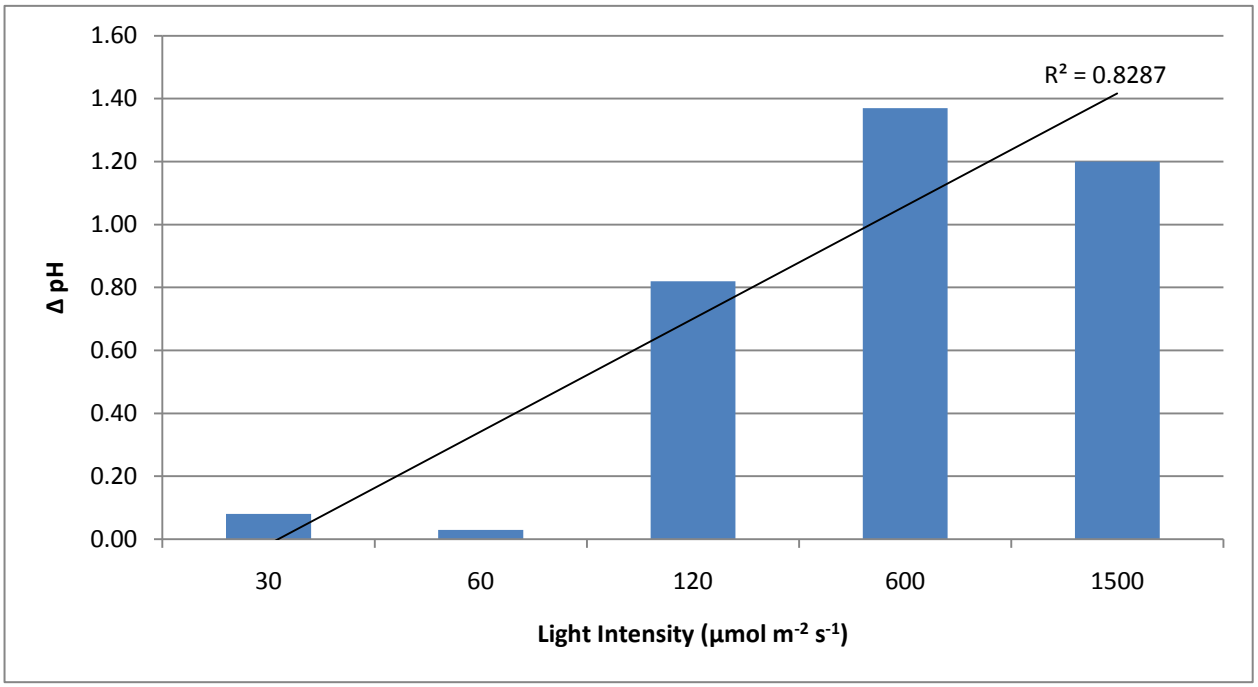

Figure 18: Showing the relationship between Δ pH and different light intensities after 14 days.

The Δ pH at a lower light intensity of 30 µmol m^{-2} s^{-1} was 0.08 units. When the light intensity was increased to 120 and 600 µmol m^{-2} s^{-1} the Δ pH increased by 0.82 and 1.37 units respectively. However although the Δ pH was fairly high at the maximum light intensity of 1500 µmol m^{-2} s^{-1} there was a slight decline when compared to that observed at a light intensity of 600 µmol m^{-2} s^{-1}.

There was also an observable relationship between Δ pH and the dry biomass formation. This relationship seemed to have been influenced by the light intensity as shown in figure 19 below.

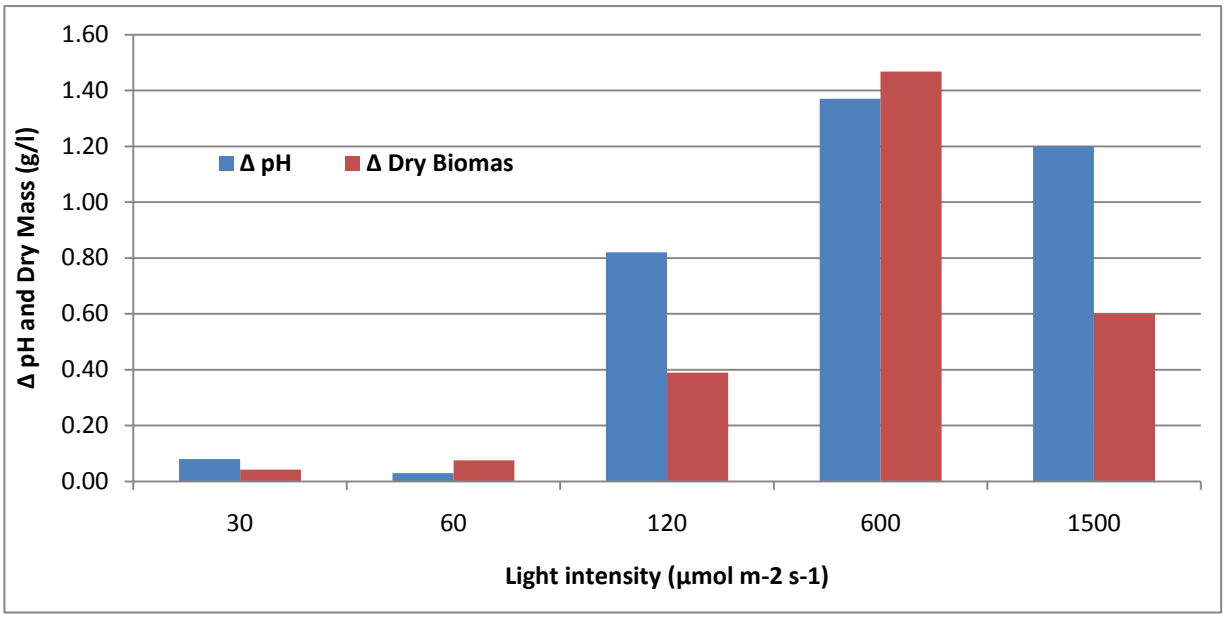

Figure 19: Showing the relationship between Δ pH and biomass at different light intensity.

The results seem to indicate that whenever there was an increase in Δ pH there was an observable increase in Δ dry biomass formation but this was dependent on the light intensity.

At this point the results seem to indicate that even though the relationship between light and biomass formation is directly proportional there is a threshold beyond which no increase in biomass formation takes place. These results also seem to also indicate that light intensity had a great influence on the growth rate of the algal culture in the bioreactor that could have also influenced biomass formation and change in pH.

4.5. Effect of different regimens of light/ dark cycle treatment on biomass formation in the novel tubular photobioreactor

Light/ dark cycles have been reported (Grobbelaar 1991; Terry 1986) to greatly influence the formation of algal biomass. Therefore it was important to evaluate the growth of *Spirulina* in the novel tubular photobioreactor under varying conditions of light/ dark cycles using both natural and artificial lights so as to achieve the aim of the project. Based on the observations

made regarding the effect of light intensity on the biomass formation of *Spirulina* in the photobioreactor, three light intensity regimes treatments were used to evaluate the effect of light/ dark cycles on biomass formation of *Spirulina*. These were 120 µmol m^{-2} s^{-1} which was the maximum intensity emitted by fluorescent light tubes, 600 µmol m^{-2} s^{-1} the maximum emitted by LED light and finally 1500 µmol m^{-2} s^{-1} which was the maximum light intensity received from the sun at the point of exposure during summer. It is important to understand at this point that the flow of the algal culture between the dark phase (where darkness was experienced) and light phase (where light was experienced) was constant and was determined by the diaphragm pump.

4.5.1. Effect of differing light/ dark ratios at 120 µmol m^{-2} s^{-1}

Two light/ dark ratios were used to study the effect that a light intensity of 120 µmol m^{-2} s^{-1} might have had on algal biomass formation i.e. a ratio of 1:1 where half the time the algal culture experienced darkness and half the time it experienced light and then 1:1.4 where 1 part of time light was experienced and 1.4 parts of the algal culture volume at any given moment experienced darkness.

At a light intensity of 120 µmol m^{-2} s^{-1} it was observed that more algal biomass formation was achieved when the algal culture had been cultivated at a light/ dark ratio of 1:1 when compared to that of 1:1.4 (figure 20).

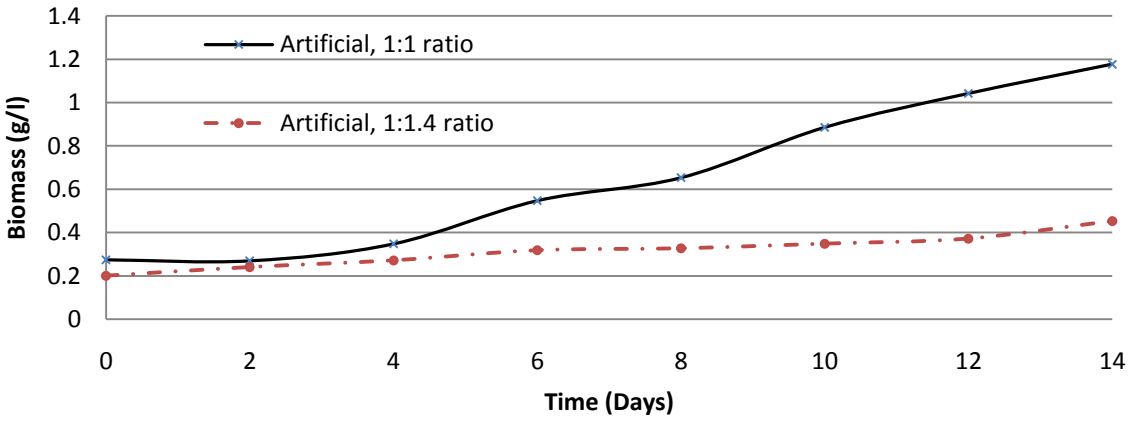

Figure 20: Showing the maximum biomass produced when the novel tubular photobioreactor was operated at a light intensity 120 µmol m-2 s-1 at a ratio of 1:1 and 1:1.4 respectively.

The maximum change in dry biomass formation when a light/ dark cycle of 1:1 was used was 0.90g/l when compared to that obtained at a light/ dark cycle ratio of 1:1.4 which was 0.17g/l. These results seemed to indicate that a longer time spent in the dark would not result in higher biomass formation. Furthermore the specific growth rate of the algal culture at a light/ dark ratio of 1:1 was 0.9289 while that at a ratio of 1:1.4 was 0.205. It was observed that there was a slightly higher change in pH (0.59 units) of the culture run at a light/ dark cycle ratio of 1:1 as opposed to that run at a ratio of 1:1.4 (0.41 units).

4.5 2. Effect of differing light/ dark ratios at 1500 µmol m^{-2} s^{-1}

At a light intensity of 1500 µmol m^{-2} s^{-1} a similar light/ dark cycle ratio as that used previously (at 120 µmol m^{-2} s^{-1}) was used. It was observed that there was a greater increase in biomass formation when the photobioreactor was illuminated with 1500 µmol m^{-2} s^{-1} (figure 21) as opposed to a light intensity of 120 µmol m^{-2} s^{-1}.

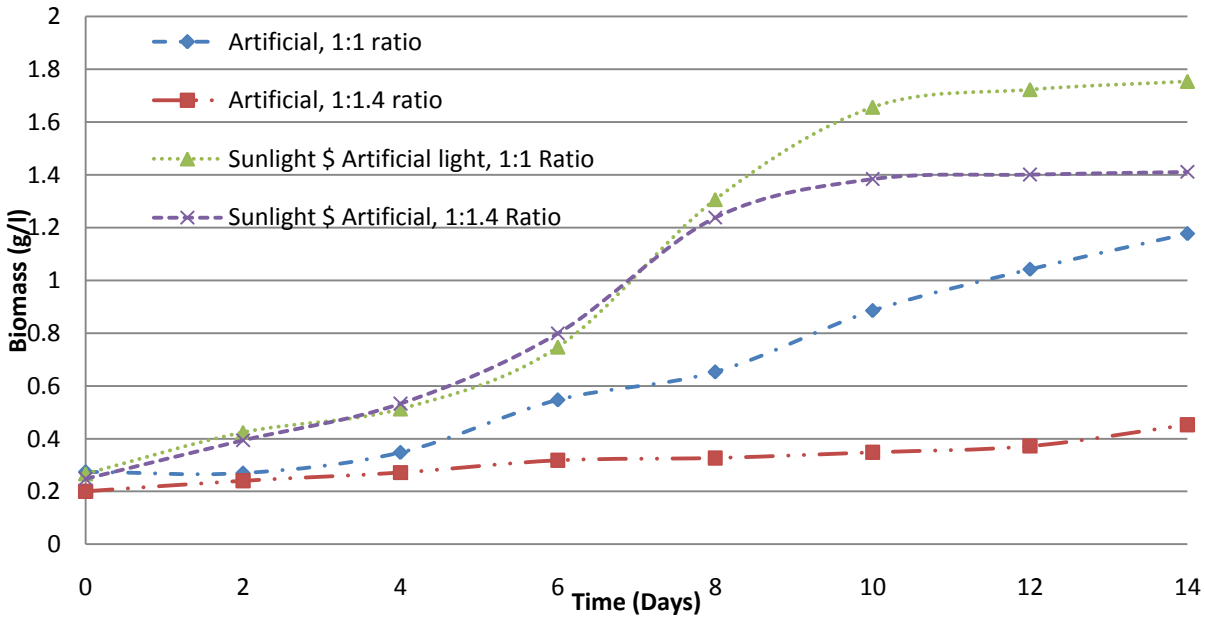

Figure 21: : Showing a comparison of biomass produced at a ratio of 1:1 and 1:1.4 when the novel tubular photobioreactor was operated at a light intensity of 120 and 1500 µmol m-2 s-1 respectively.

At a light/ dark cycle ratio of 1:1 the change in dry biomass formation was 1.49g/l while at a ratio of 1:1.4 the observable change in dry biomass formation was 1.16g/l. The specific growth rate of the algal cells in the culture treated with a light/ dark ratio of 1:1 was 1.506 while that treated with a light/ dark ratio of 1:1.4 was 1.163. A higher pH difference of 1.2 units was also observed in the algal culture treated with a light/ dark cycle ratio of 1:1 when compared to that treated with a light/ dark cycle ratio of 1:1.4 which was 0.44 units.

4.5.3. Effect of differing light/ dark ratios at 600 µmol m^{-2} s^{-1}

Following the results obtained on the effect of light on the algal biomass formation, it was observed that of all the light intensities used 600 µmol m^{-2} s^{-1} offered better light conditions that favoured a greater biomass formation. The effects of four different light/ dark cycle ratio treatments were used i.e 1:0, 1:0.25, 1:1, 1:1.5 and 1:2. The cultivation of the *Spirulina* under all these different light/ dark cycle ratios resulted in a much higher biomass yield when compared to that obtained when the photobioreactor was illuminated with a light intensity of 120 µmol m^{-2} s^{-1} and 1500 µmol m^{-2} s^{-1} (figure 21). These results showed that when the algal cells spent more time in the light they reached their maximum biomass yield much sooner. For instance it took 7 to 8 days for cells to reach a maximum biomass formation of 2.1g/l and 2.15g/l when treated at a light/ dark cycle ratio of 1:0 and 1:0.25 respectively. But after 8 days of exposure a sharp decline in biomass formation was observed and was preceded by a drastic colour change in the algal culture from green to yellow and finally brown towards the end of the 14 days. On the other hand the results seemed to indicate that there was a relationship between biomass formation and the light/ dark cycle ratio (figure 22). It was observed that the amount of time taken for the algal culture to reach the maximum biomass was less with reduced time spent in the dark (figure 23).

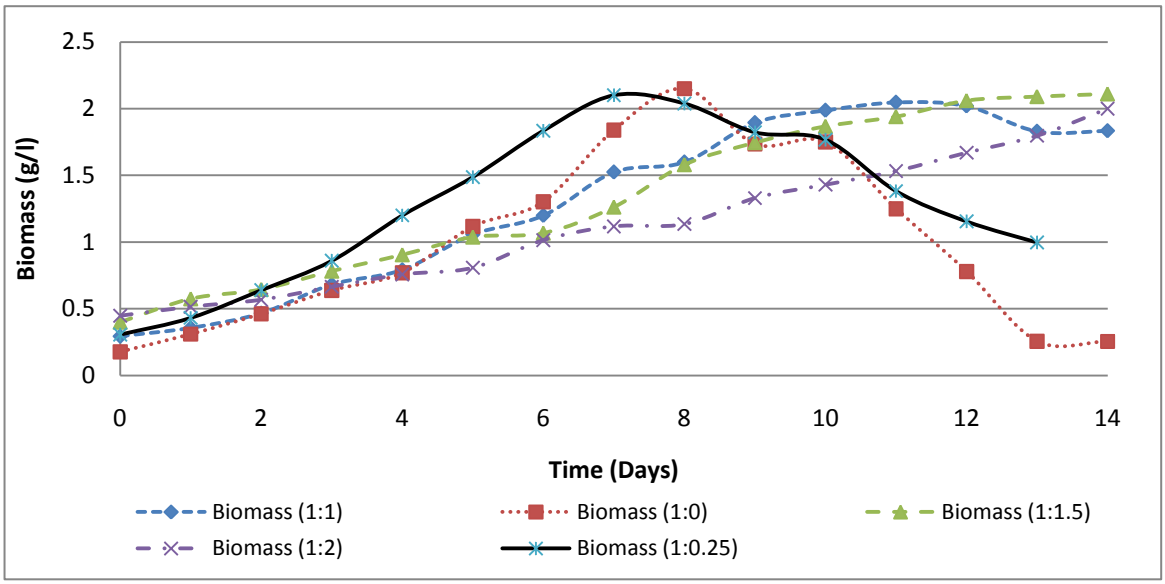

Figure 22: Showing maximum biomass produced at different light/ dark ratios when the novel tubular photobioreactor was operated at a light intensity of 600 µmol m-2 s-1.

The greatest increase in biomass formation was achieved after a period of 7 days (figures 22 and 24). When the values of biomass formation from all the different treatments at the same time point were taken and compared an inversely proportional relationship between light/ dark ratio and biomass formation was seen.

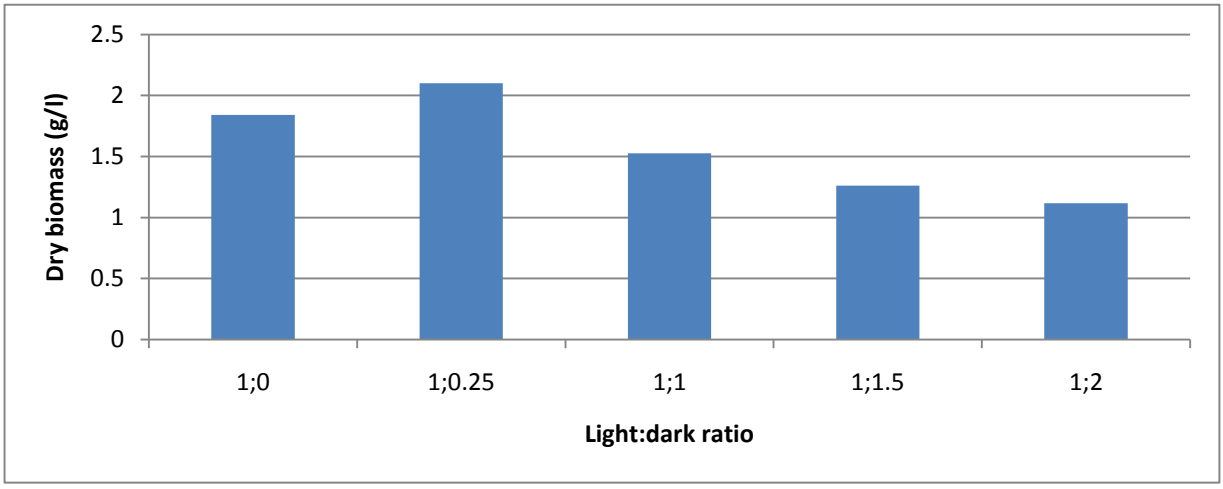

Figure 23: Showing the maximum biomass produced at different light/ dark ratios when the novel tubular photobioreactor was operated a light intensity of 600 µmol m-2 s-1 for 7 days.

During these experiments the pH was continuously monitored and there was an observable relationship between pH and biomass formation at the different light/ dark cycle treatment ratios. The pH was seen to increase with an increase in dry biomass until a maximum of the biomass formation was reached (figure 24). It was noticed that the biomass started to decrease whenever a critical pH of 12 was reached.

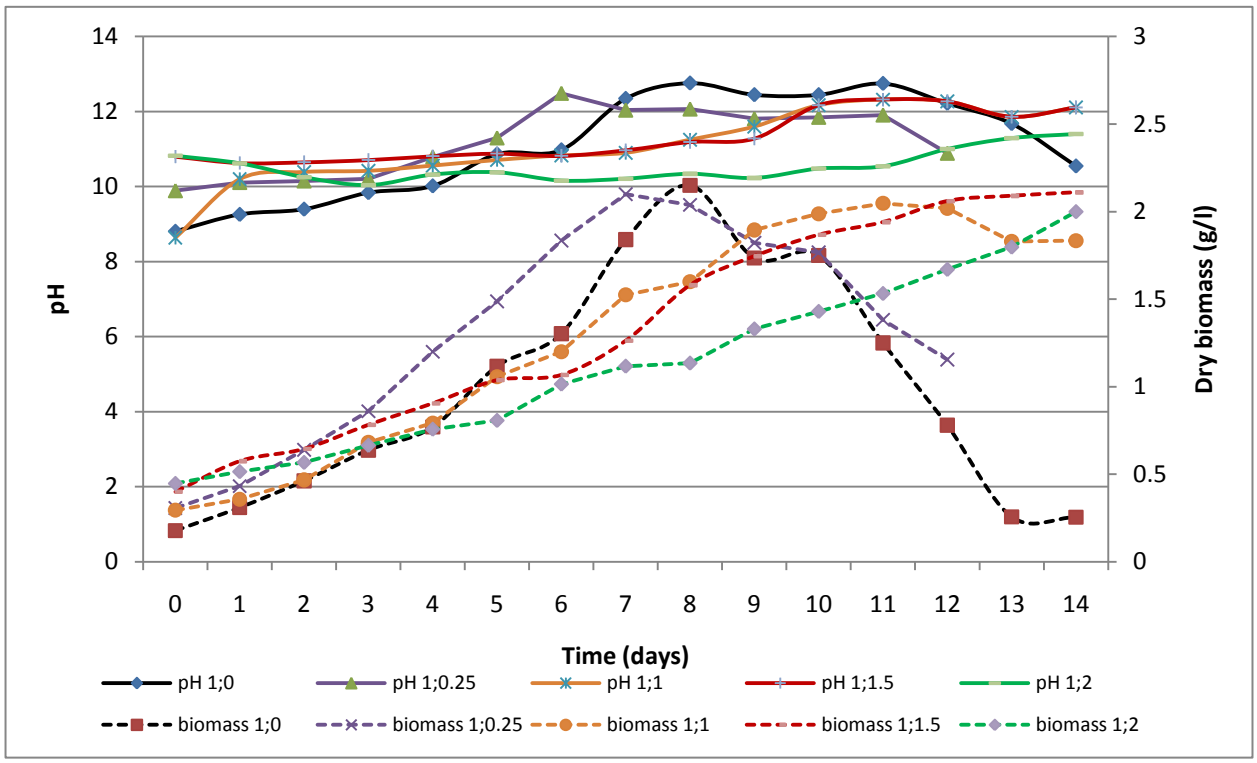

Figure 24: Showing the relationship between dry biomass formation and pH.

4.6 Operation of the novel photobioreactor as a continuous system

Following the evaluation of the effect of light/ dark cycle ratios under the different settings stated above the operation of the photobioreactor as a continuous system was initiated. The continuous system mode of operation was done indoors with LED light emitting a maximum light intensity of 600 µmol m^{-2} s^{-1}.

The photobioreactor was started as a batch and was operated at a light/ dark ratio of 1:0.25. The maximum biomass produced during the continuous cultivation of *Spirulina* using LED light was 32 g/l/day (figure 25).

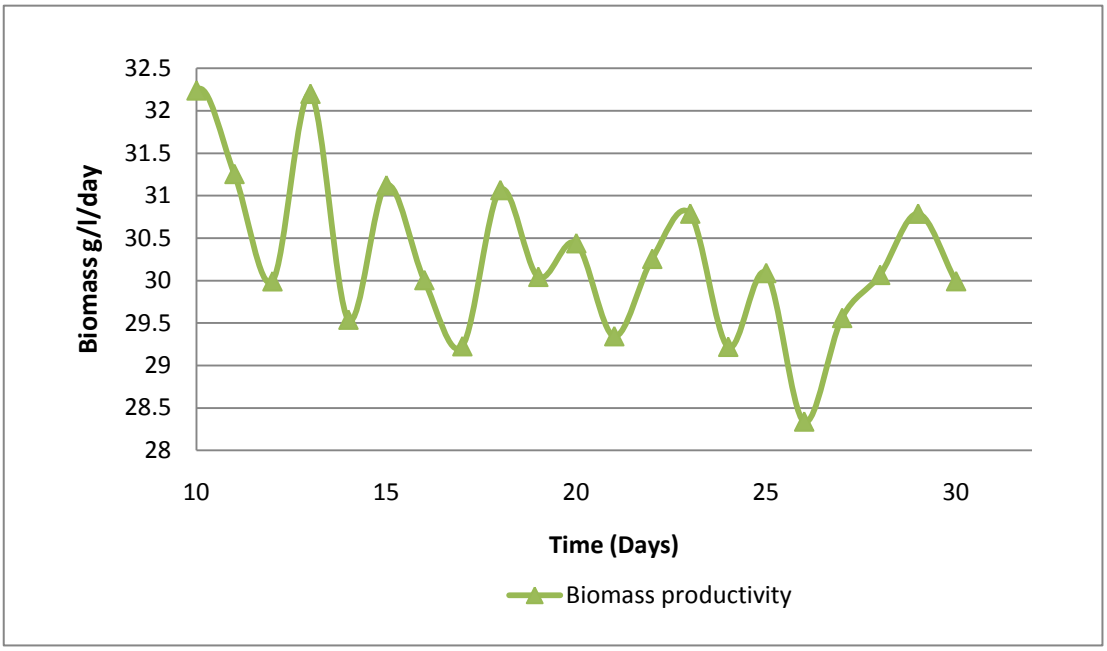

Figure 25: Showing the continuous cultivation of Spirulina on a novel tubular photobioreactor at a light intensity of 600 µmol m-2 s-1using a turbidostat.

CHAPTER 5

5. DISCUSSION

5.1. Introduction

This study was aimed at the construction and operation of a novel photobioreactor towards the optimization of conditions required for algal biomass production. The alga *Spirulina platensis* was chosen as the alga that would be used to determine the ability of the constructed novel tubular photobioreactor in optimizing algal biomass production. Before *Spirulina platensis* could be grown in the novel tubular photobioreactor, its growth curve had to be generated in a controlled environment (laboratory). This was then followed by the growth of *Spirulina* in a novel tubular photobioreactor at different light intensities with the aim of determining the optimum light intensity which could be used to cultivate *Spirulina.* After the optimization of light, *Spirulina* was then grown in a novel tubular photobioreactor which was operated in a batch mode system at different light/ dark ratios in order to determine the light: dark ratio which yields the highest biomass productivity. The best light/ dark ratio was then chosen and used to cultivate *Spirulina* in the novel tubular photobioreactor on a continuous mode system.

5.2. The construction of the photobioreactor

The novel tubular photobioreactor was constructed in such a way that it consisted of two parts i.e. one part which allowed the algal culture (*Spirulina)* to be illuminated, either by sunlight or natural light or both and the second part which was completely dark. This photobioreactor was constructed to have both light and dark phases so that it could be used to achieve the objective of evaluating the effect of varying light/ dark ratios on algal biomass productivity.

The light phase of the reactor was constructed in a form of a pyramid-like structure. The pyramid-like shape was chosen for the light phase of the photobioreactor for its ability to maximize light exposure. Light has been reported as a critical limiting factor during the operation of photobioreactors (Janssen *et al*. 2000; Janssen *et al*. 2001a) and due to this, the photobioreactor had to be designed in such as way as to optimize efficient light exposure. The light phase of the reactor was made up of clear PVC tubing which allowed for the transition of

PAR either from artificial lights fitted within this phase of the work or sunlight. PAR is important because this is the radiation spectrum that can be utilised by algae. The light phase of the reactor was classified as a tubular photobioreactor because it was made up of a tube which was exposed to light. Tubular photobioreactors in relation to other reactors are known to be more suitable for commercial production of algae due to their large illumination surface area (Ugwu & Aoyagi 2008) hence a tubular photobioreactor was chosen to make up the light phase of this novel photobioreactor.

The dark phase of the photobioreactor was encapsulated in an opaque box to which the light phase was attached. The opaque box contained a diaphragm pump which was connected to a number of reservoirs. This diaphragm pump was responsible for circulating the algal cells through the two phases (light and dark). A diaphragm pump was chosen for this study because unlike other pumps, its piston action allows it to smoothly pump the algal cells in and out without breaking the cells.

The light and dark phases of the reactor were then joined together to make up a novel tubular photobioreactor which has the ability to expose the cultured alga to a light/ dark phase (light/ dark cycle). A provisional as well as an international patent of the photobioreactor was approved (appendix).

5.3. Evaluation of growth conditions of the inoculum

The alga *Spirulina platensis* was chosen as a test organism for this study because of a number of reasons such as its tolerance of high pH and temperature values which limits contamination, the ability to use bicarbonate as a source of carbon which reduces the cost of operation by not using gaseous carbon dioxide, its bigger cells which makes them cheaper to harvest and finally its global socioeconomic importance.

Before the alga could be cultivated in the novel tubular photobioreactor, it was essential to establish an inoculum. The algal inoculum was established in the laboratory using Schlösser liquid growth medium. Schlösser liquid growth medium was used in this study because it

contains all the nutrient ingredients required for the growth of *Spirulina* as stated in section 2.11 of the introduction and most importantly, it was the growth medium that the alga had previously been adapted to. It was also the recommended liquid growth medium according to the supplier of the algal culture (University of North West, South Africa). A growth curve of the alga in this medium was determined for a period of 18 days.

The results obtained seemed to indicate that Schlösser liquid growth medium was suitable for the growth of *Spirulina*. The alga showed an exponential increase in growth as indicated by both the OD_{680nm} and biomass readings although most of the growth curve was mainly made up of the log phase and an early stationary phase (figure 14). The absence of a lag phase in the growth curve can be attributed to the fact the alga did not have to adapt to the growth medium since it was the growth medium that it was previously maintained on by the culture supplier. This means that the alga did not have to synthesize key enzymes and molecules that are necessary to metabolize the substrates present in the medium. The length of the lag phase can also depend on a variety of factors such as the size of the inoculum, time necessary to recover from physical damage or shock encountered during the transfer and time required to synthesize essential coenzymes or division factors. The reason for an early stationary phase can be attributed to the absence of a lag phase, which means that all the nutrients could have been quickly used up. This phase is often characterised by the exhaustion of the available nutrients, accumulation of inhibitory metabolites or by-products and exhaustion of space.

Parameters such as temperature and pH were also monitored during the evaluation of the growth conditions of the inoculum. Temperature was always found to be between 25 and 30 ^{o}C as shown in figure 15. Although this was not the optimum temperature that the alga could grow at (Binaghi *et al.* 2003), this temperature seemed to be sufficient to support good algal growth. The pH increased from the initial pH which was 9.3 to 10 (figure 15). *Spirulina* uses bicarbonate (HCO_3^-) as a source of carbon by converting it to carbon dioxide (CO_2) and hydroxide ion (OH^-) during photosynthesis (Reuter & Müller 1993; Jaiswal *et al*. 2005; Badger *et al*. 1994; Badger& Price 2003). The gradual increase in pH in this case could have been caused by the accumulation of OH^- which resulted from the fixation of CO_2 by the photosynthetic process. The tendency of the pH to rise has also been reported by Andrade & Costa (2007) to be

related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher. Although there was an observable increase in pH, the final pH was still well within *Spirulina*'s pH limit of 11 (Binaghi *et al*. 2003) hence a good growth of this organism was observed at this pH condition.

5.4. The effect of light intensity on the growth of *Spirulina platensis* in the novel tubular photobioreactor.

In order to optimize the novel tubular photobioreactor for optimal algal biomass productivity, it was important to determine the effect that light intensity had on the biomass production of this alga. This was done by subjecting the alga to three different light intensities (120, 60 and 30 µmol m^{-2} s^{-1}) for the same duration. A higher biomass of 1.2 g/l was produced at a light intensity of 120 µmol m^{-2} s^{-1} when compared to 0.68 and 0.38 g/l produced at light intensities of 60 and 30 µmol m^{-2} s^{-1} respectively. These results seemed to indicate that there was a direct proportional relationship between light intensity and biomass formation as shown in figure 16. The results also seemed to support those reported by Oncel & Akpolat (2006) which suggested that the growth rate of microalgae increases in proportion with increasing light intensity until saturation levels are reached. Saturation level is a threshold beyond which an increase in light intensity does not lead to an increase in biomass formation.

The highest specific growth rate of 1.207 was achieved at a light intensity of 120 µmol m^{-2} s^{-1} when compared with 0.516 and 0.204 achieved at light intensities of 60 and 30 µmol m^{-2} s^{-1} respectively. The fact that the highest biomass and specific growth rate was achieved at the maximum light intensity of florescent lights seemed to suggested that an increase in light intensity would still result in an increase in biomass formation. This meant that saturation levels had not been reached hence light intensity was increased to 600 µmol m^{-2} s^{-1} with the use of LED lights.

When LED lights known to emit a much higher light intensity (600 µmol m^{-2} s^{-1}) than fluorescent lights were used, about 2.38 g/l of biomass was produced which was almost double that which was produced at maximum fluorescent lights. However, the increase in biomass productivity was not directly proportional to the increase in light intensity as previously observed. This result seemed to suggest that saturation levels could have been reached. To confirm whether saturation levels had in fact been reached, the algal culture was subjected to a much higher light intensity of sunlight (maximum was found to be 1500 µmol m^{-2} s^{-1}) during the day and a low light intensity of fluorescent tubes during the night (120 µmol m^{-2} s^{-1}). However, the results obtained when the photobioreactor was operated with a combination of sunlight and fluorescent tubes, no further increase in algal biomass production was observed when compared to that achieved when the photobioreactor was illuminated by LED lights alone (figure 17). This shows that saturation levels had indeed been achieved. These results seemed to support the results published by Sorokin & Krauss (1965) which suggest that an increase in light intensity first favours cell division then, once the optimal light intensity is attained, a further increase in light intensity inhibits the cell division process.

Parameters such as temperature and pH were also monitored during the investigation of the effect that light intensity could have had on biomass formation. When the novel tubular photobioreactor was operated in the laboratory, temperature was kept constant at its optimum (36 ^{o}C ± 2 ^{o}C) by heating the room with external heaters. Different temperature values were reported when the photobioreactor was operated using sunlight. The difference in temperature values was due to variation in the ambient temperature.

The highest change in pH of 1.37 was reported when the photobioreactor was operated using LED lights (600 µmol m^{-2} s^{-1}) and the lowest was reported when fluorescent lights were used (at a light intensity of 60 µmol m^{-2} s^{-1}) (figure 17). The lowest change in pH was reported at a light intensity of 60 µmol m^{-2} s^{-1} as opposed to 30 µmol m^{-2} s^{-1}; this could have been due to an error in the pH probe. Andrade & Costa (2007) reported that the tendency of the pH to rise was related to *Spirulina*'s photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher. According to the results obtained the change in pH at 30 µmol m^{-2} s^{-1} was higher than at 60 µmol m^{-2} s^{-1}, but figure 19 shows that there was higher biomass

productivity (meaning there was higher activity) at 60 µmol m^{-2} s^{-1} than at 30 µmol m^{-2} s^{-} . This justifies the fact that there was an error in the pH probe when the photobioreactor was being operated at 60 µmol m^{-2} s^{-1}.

Figure 19 also shows that there was a direct relationship between change in pH, biomass productivity and light intensity until saturation level was reached. This implied that the higher the light intensity the greater the photosynthetic activity and the higher the photosynthetic activity the more biomass produced until saturation level was reached. Saturation level seemed to have been reached when light intensity was at 600 µmol m^{-2} s^{-1}.

5.5. Effect of different regimens of light/ dark cycle treatment on biomass formation in the novel tubular photobioreactor

The novel tubular photobioreactor was constructed to have both the light and dark phases so that it could be used in evaluating the effect of the light/ dark cycle on algal biomass productivity which has been reported to greatly enhanced biomass productivity (Grobbelaar 1991; Terry 1986). The light/ dark cycle was evaluated at three different light intensities, namely 120, 600 and 1500 µmol m^{-2} s^{-1}.

5.5.1. Effect of differing light/ dark ratios at 120 µmol m^{-2} s^{-1}

The two light/ dark ratios used at this light intensity were 1:1 and 1:1.4 respectively. The highest biomass formation was produced at a light/ dark ratio of 1:1 when compared to that obtained at a ratio of 1:1.4 (figure 20). These results seem to indicate that the photosynthetic activity was higher when the alga was spending equal amounts of time in light and dark phases as compared to when it was spending more time in the dark relative to the light phase. A higher specific growth rate of 0.9298 and change in pH of 0.59 was obtained at a light/ dark ratio of 1:1 when compared to a specific growth rate of 0.208 and a change in pH of 0.17 achieved at a light/ dark ratio of 1:1.4. An increase in pH has been found to be directly related to an increase in photosynthetic activity hence a higher specific growth rate was achieved at a light/ dark ratio 1:1, where a higher change in pH was reported. The results seemed to support those reported by Grobbelaar *et al*. (1996) which suggested that a longer dark period relative to the preceding light period does not necessary lead to higher biomass productivity.

5.5.2. Effect of differing light/ dark ratios at 1500 µmol m^{-2} s^{-1}

The same ratios used when the light intensity was 120 µmol m^{-2} s^{-1} were also used at a light intensity of 1500 µmol m^{-2} s^{-1}. A higher maximum biomass of 1.78 g/l was produced at a light/ dark ratio of 1:1 when compared to 1.45 g/l produced at a light/ dark ratio of 1:1.4 (figure 21). These results seem to suggest that the photosynthetic rate was higher when the alga was spending equal amounts of time in light and dark phases (1:1) as compared to when the alga was spending more time in the dark phase (1:1.4). However, maximum biomasses produced at this light intensity, in both the light/ dark ratios used were found to be much higher than when compared with those produced at the same ratios but at a light intensity of 120 µmol m^{-2} s^{-1} (figure 21). This meant that the light intensity of 1500 µmol m^{-2} s^{-1} provided a better light condition which promoted higher cell growth when compared to that provided by a light intensity of 120 µmol m^{-2} s^{-1}. However, it was found that in both light intensities (120 and 1500 µmol m^{-2} s^{-1}), the light/ dark ratio of 1:1 produced better results when compared to those produced at a ratio of 1:1.4. These results suggest that a longer dark cycle relative to light does not result in higher biomass production as reported by Grobbelaar *et al*. (1996).

Although the growth curves for the two light/ dark ratios (1:1 and 1:1.4) were generated for a period of 14 days using both light intensities (120 and 1500 µmol m^{-2} s^{-1}), the growth curves at a light intensity of 1500 µmol m^{-2} s^{-1} had a well defined stationary phase when compared to those generated at a light intensity of 120 µmol m^{-2} s^{-1}. The growth curves at a light intensity of 120 µmol m^{-2} s^{-1} were still at their exponential phase even after a period of 14 days as shown in figure 21. These growth curves (for a ratio of 1:1 and 1:1.14 at a light intensity of 120 µmol m^{-2} s^{-1}) also had lower specific growth rates of 0.9 (1:1) and 0.208 (1:1.4) when compared with 1.506 and 1.163 obtained when a light intensity of 1500 µmol m^{-2} s^{-1} was used.

Lower changes in pH (Δ pH) of 0.59 and 0.41 were also observed at the ratios of 1:1 and 1:1.4 respectively when a light intensity of 120 µmol m^{-2} s^{-1} was used compared to 1.2 and 0.44 reported at the two light/ dark ratios when the light intensity of 1500 µmol m^{-2} s^{-1} was used. The lower specific growth rate and change in pH at a light intensity of 120 µmol m^{-2} s^{-1} resulted

in a prolonged exponential phase which suggested that the light condition provided by this light intensity limited the algal growth.

Section 5.5.1 and 5.5.2 of the discussion indicated that the light intensity of 120 µmol m^{-2} s^{-1} was not enough to be able to promote good algal growth hence the algal growth was limited at this intensity whereas the light intensity at 1500 µmol m^{-2} s^{-1} (light intensity provided by sunlight) was able to promote good algal growth but the amount of light intensity from sunlight varied depending on the cloud cover. The next step in this study was to operate the novel tubular photobioreactor using high intensity artificial lights under controlled conditions (in the laboratory). Therefore, the work was conducted at a light intensity of 600 µmol m^{-2} s^{-1} using LED lights.

5.5.3. Effect of differing light/ dark ratios at 600 µmol m^{-2} s^{-1}

The light/ dark ratios evaluated at a light intensity of 600 µmol m^{-2} s^{-1} were 1:0, 1:0.25, 1:1, 1:1.5 and 1:2. The results showed that all the light/ dark ratios used at this light intensity produced higher biomasses when compared to the light/ dark ratios used at a light intensity of 120 and 1500 µmol m^{-2} s^{-1}. The higher biomasses produced in all the ratios at this light intensity can be attributed to the fact that this light condition was found to be the best when compared to other in section 5.4 of the discussion. This light intensity was also found to be the light condition at which light saturation levels were reached according to section 5.4 of the discussion.

The results obtained showed that all the light/ dark ratios (five) evaluated at this light intensity achieved the same maximum biomass (figure 22) of 2.3 g/l. This suggests that the maximum biomass produced by the different light/ dark ratios was the maximum amount that the alga (*Spirulina*) could produce under the novel tubular photobioreactor's operating conditions (light, temperature, pH and turbulence etc.) regardless of the light/ dark ratio used. However, the crux in the different light/ dark ratios seemed to have been the amount of time it took to reach the maximum biomass that could be produced by the alga. The ratio that produced this maximum biomass in the shortest period of time was the ratio of 1:0.25 followed by the ratio of 1:0 (light

only exposure). The results suggest that the ratio of 1:0.25 needed more light and only a small amount of darkness for maximum algal biomass production.

The light/ dark ratio of 1:0.25, which means 2.0 min in light and 30 sec in dark (which yielded the highest biomass in the shortest period of time) can be categorized under the medium frequency fluctuations according to the light/ dark frequency fluctuation category developed by Grobbelaar (1989). Grobbelaar (1989) categorized the frequency fluctuations as high (>10 Hz), medium (seconds to minutes) and low (hours to days). Of these three, high frequency fluctuation has been reported to increase the rate of photosynthetic efficiency (Terry 1986; Grobbelaar 1989; Grobbelaar *et al*. 1996a) whereas low frequency fluctuation has been reported to influence algal cell division during photosynthesis as well as their circadian rhythm activity. On the other hand, according to Grobbelaar (1989) medium frequency fluctuation is associated with turbulence within the algal culture. Although there is not enough data on medium frequency fluctuations, Grobbelaar (1989) seems to suggest that turbulence may influence photosynthetic efficiency hence the increase in biomass productivity during this medium frequency fluctuation.

When longer dark cycles relative to light cycles were used, the rate of photosynthesis decreased as shown by the longer period it took to reach the maximum algal biomass production (figure 22). These results support those published by Terry (1986) who reported that below or above a certain light/ dark ratio the rate of photosynthesis and culture productivity declines.

Figure 23 indicates that there was an inversely proportional relationship between biomass production and light/ dark ratio, which meant that the lower the light/ dark ratio (1:0.25) the higher the photosynthetic activity whereas the higher the light/ dark ratio (1:2) the lower the photosynthetic activity. These results also support those reported by Grobbelaar *et al*. (1996) which suggested that longer dark period relative to the preceding light period does not necessary lead to higher biomass productivity. Figure 23 showed that the rate of the biomass production was directly proportional to an increase in pH. The increase in pH in *Spirulina* culture was caused by the accumulation of OH^- which resulted from the fixation of CO_2 by the

photosynthetic process (conversion of bicarbonate into carbon dioxide and hydroxide ion). The tendency of pH to rise has also been reported by Andrade & Costa (2007) to be related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher. It was also observed that at a ratio of 1:0 and 1:0.25 the biomass production declined after 8 and 7 days respectively. Binaghi *et al*. (2003) reported that biomass productivity of *Spirulina* decreases in environments with pH values higher than 11, hence a sharp decline in biomass productivity when the pH reached 12 (figure 24) at the ratio of 1:0 and 1:0.25.

A study to detect the effect of differing light/ dark ratios at a light intensity of 600 µmol m^{-2} s^{-1} was conducted in a novel tubular photobioreactor using a batch mode system of operation. Maximum algal biomass production was achieved (in the shortest period of time) at a light/ dark ratio of 1:0.25 at a pH below 12. This lead to the use of these growth conditions in the novel tubular photobioreactor under continuous culture cultivation. Continuous cultivation was achieved by incorporating a turbidostat to control the biomass production and the pH.

5.6. Operation of the novel photobioreactor as a continuous system

A continuous mode of operation of the novel tubular photobioreactor was conducted in the laboratory at a light/ dark ratio of 1:0.25 and a light intensity of 600 µmol m^{-2} s^{-1}. The continuous cultivation mode was achieved by the use of a turbidostat (Pap´aˇcek 2005). The maximum biomass produced during this continuous cultivation was found to be 32 g/l/day (25 g/m^2/day) (figure 25) and this biomass productivity corresponded to a net photosynthetic efficiency of 5.7 %. This result was found to be higher than what most photobioreactors would produce when *Spirulina* is cultivated (Richmond 2004). However, the results were found to be slightly lower than the 27.8 g/m^2/day reported when a two-plane tubular photobioreactor was used by Torzillo *et al*. (1993). The 2.8 g/m^2/day difference in biomass productivity between the two photobioreactors can be attributed to the different materials used in the construction of these photobioreactors. The two-plane tubular photobioreactor was constructed using clear glass whereas the novel tubular photobioreactor was constructed using a clear PVC tube. PVC tubes are known to be affected by environmental parameters such as higher temperature and

pH values which can affect the clarity or transparency of the tube and hence can block a certain amount of light. The two-plane tubular photobioreactor was operated using sunlight whereas the novel tubular photobioreactor was operated using LED lights at a light intensity of 600 µmol m^{-2} s^{-1}. Although LED lights have been demonstrated in this study to give better results than sunlight (section 5.4 of the discussion), the difference in the biomass produced in these two photobioreactors can also be attributed to the different light sources used.

5.7. Summary of the discussion

A novel tubular photobioreactor which had a light and a dark phase was constructed and patented. The alga *Spirulina* was used as a test organism in this novel tubular photobioreactor. The best light condition that favored higher *Spirulina* biomass formation in the novel tubular photobioreactor was found at a light intensity of 600 µmol m^{-2} s^{-1}. This light intensity was also found to be the light intensity at which saturation levels were reached. When different light/ dark ratios were evaluated with respect to biomass production at a light intensity of 600 µmol m^{-2} s^{-1}, it was found that longer light cycles relative to dark cycles result in higher biomass production. A maximum biomass productivity of 25 g/m^2/day was achieved when the novel tubular photobioreactor was operated using longer light cycles (1:0.25) relative to dark cycles. This biomass productivity corresponded to a net photosynthetic efficiency of 5.7 %. This result was found to be higher than what most photobioreactors produce but slightly lower than the highest ever reported productivity in a photobioreactor.

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The aim of this study was to develop a novel tubular photobioreactor and use it to optimize the production of *Spirulina* biomass. In order to achieve this aim, a number of objectives were set as outlined in chapter 1. This was followed by the accumulation of data through experiments and results obtained were reported and interpreted in chapter 4 and 5 respectively.

6.1.1. The construction of the photobioreactor

A novel tubular photobioreactor with a light and a dark phase was successfully constructed. A provisional and an international patent of this novel tubular photobioreactor was obtained/approved.

6.1.2. Evaluation of growth conditions of the inoculum

The alga *Spirulina platensis* was chosen as a test organism, and was used to evaluate the ability of the novel tubular photobioreactor in optimizing algal biomass production. This organism was successfully cultivated (at laboratory scale) using Schlösser liquid growth medium.

6.1.3. The effect of light intensity on the growth of *Spirulina platensis* in the novel tubular photobioreactor

Five different light intensities were evaluated, and it was found that the best light condition that favoured higher biomass formation was at 600 µmol m^{-2} s^{-1} light intensity. A direct proportional relationship between biomass production and light intensity was observed before saturation levels could be reached. This proportional relationship was also observed between Δ pH and biomass production but it was dependent on light intensity.

6.1.4. Effect of different regimens of light/dark cycle treatment on biomass formation in the novel tubular photobioreactor

The effect of light/dark cycles on *Spirulina* biomass productivity was evaluated using three different light intensities, namely 120, 600 and 1500 µmol m^{-2} s^{-1}.

6.1.4.1. Effect of differing light/dark ratios at 120 and 1500 µmol m^{-2} s^{-1}

Two light/dark ratios were used in these light intensities and it was found that the ratio of 1:1 produced higher biomass when compared to the ratio of 1:1.4. The results also showed that a longer dark cycle relative to light does not result in higher biomass production at these light intensities.

6.1.4.2. Effect of differing light/dark ratios at 600 µmol m^{-2} s^{-1}

Five different light/ dark ratios were evaluated in this light intensity and all of them produced the same maximum biomass. The most important factor was found to be the time it took for each of the light/ dark ratio to reach the maximum biomass. The light/ dark ratio of 1:0.25 was found to be most efficient ratio as it produced the maximum biomass in the shortest period of time when compared to other ratios. The results also showed that a shorter dark cycle relative to light cycles results in higher biomass production. It was also observed that pH values above 12 result in a decline in *Spirulina*'s photosynthetic activity and ultimately lead to culture death.

6.1.5. Operation of the novel photobioreactor as a continuous system

The novel tubular photobioreactor was operated as a continuous system at a light/ dark ratio of 1:0.25 and a light intensity of 600 µmol m^{-2} s^{-1}. Maximum biomass productivity of 32 g/l/day (equivalent to 25 g/m^2/day) was achieved which is higher than what most other photobioreactors have been reported to produce by Richmond (2004). The results were also found to be 2.8 g/m^2/day lower than the highest biomass production ever reported in a tubular photobioreactor (Richmond 2004).

6.2 Recommendations

1. Continuous cultivation of *Spirulina* was only conducted using LED lights at a light/ dark ratio of 1:0.25 in this study, and it would therefore be important to evaluate the continuous cultivation of this organism at the similar ratio using sunlight.
2. The PVC tubing used in this study was affected by the high pH and temperature values which the photobioreactor was being operated at, hence the tube changed from its clear colour to a cloudy/milky colour which blocked a certain amount of light. It is therefore recommended that for future studies, clear PVC tubing with a longer life span needs to be used in the construction of the photobioreactor.
3. Dissolved oxygen is known to limit algal growth in tubular photobioreactors, but this parameter was never taken into consideration during this study and as such it would be important to suggest its measurement during future studies in this novel tubular photobioreactor. However, degassing can be made possible in this novel tubular photobioreactor if high amounts of dissolved oxygen are measured.
4. This study looked at the optimization of biomass production of a single algal species in the novel tubular photobioreactor, therefore more work needs to be conducted to evaluate the ability of this novel tubular photobioreactor in optimizing biomass production of other algal species.
5. In future, the use of solar as an alternative source of energy (using solar pumps and lights) needs to be evaluated in order to reduce the cost associated with the operation of the photobioreactor.
6. The novel tubular photobioreactor used in this study was a scaled-down version of a full-scale photobioreactor (1:10) and a full-scale test module needs to be constructed to prove the reactor's ability to optimize *Spirulina* biomass production in full-scale modular operations.

REFERENCE

ACIEN FERNA´NDEZ, F. G., GARCIA CAMACHO, F., SANCHEZ PEREZ, J. A., FERNANDEZ SEVILLA, J. M. & MOLINA GRIMA, E. 1998. Modeling of Biomass Productivity in Tubular Photobioreactors for Microalgal Cultures: Effects of Dilution Rate, Tube Diameter, and Solar Irradiance. *BIOTECHNOLOGY AND BIOENGINEERING,,* 58**,** 605-616.

AHSAN, M., HABIB, B. & PARVIN, M. 2008. A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. *FAO Fisheries and Aquaculture Circular.* Mymensingh, Bangladesh Agricultural University.

ALGAL BIOMASS ORGANISATION 2010. Draft Guidance Document: Algal Industry Minimum Descriptive Language.

ALOK, J. 2008. UK Announces World's Largest Algal Biofuel Project. *The Guardian.* Stanford.

ANDERSON, R. A. (Ed.) 2005. *Algal culturing techniques,* Burlington, Elsevier Academic Press.

ANDERSON, R. A., JENNIE, C. H.-C. & ANGELA, B. 1996. Algae. *Maintaining Cultures for Biotechnology and Industry.* San Diego, Academic Press.

ANDRADE, M. R. & COSTA, J. A. V. 2007. Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. *Aquaculture,* 264**,** 130-134.

BABADZHANOV, A. S., ABDUSAMATOVA, N., YUSUPOVA, F. M., FAIZULLAEVA, N., MEZHLUMYAN, L. G. & MALIKOVA, M. K. 2004. Chemical Composition of *Spirulina platensis* Cultivated in Uzbekistan. *Chemistry of Natural Compounds,* 40**,** 276-279.

BADGER, M. R., PALMQVIST, K. & YU, J. W. 1994. Measurement of CO_2 and HCO_3 fluxes in cyanobacteria and microalgae during steady state photosynthesis. *Physiologia Plantarum,* 90**,** 529-536.

BADGER, M. R. & PRICE, G. D. 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany,* 54**,** 609-622.

BECKER, E. W. & VENKATARAMAN, L. V. 1984. Production and utilization of the blue-green alga *Spirulina* in India. *Biomass,* 4**,** 105-125.

BELAY, A. 2002. *Spirulina (Arthrospira)* as a Nutritional and Therapeutic Supplement in Health Management. *The Journal of the American Nutraceutical Association,* 5**,** 27-48.

BENEMANN, J. R. 1979. Production of nitrogen fertilizer with nitrogen-fixing blue - green algae. *Enzyme and Microbial Technology,* 1**,** 83-90.

BENEMANN, J. R. 2009. Microalgae biofuels: a brief introduction. *Microalgae Biofuels,* 1**,** 1-8.

BENSON, B. C., GUTIERREZ-WING, M. T. & RUSCH, K. A. 2009. Optimization of the lighting system for a Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR): Economic implications. *Aquacultural Engineering,* 40**,** 45-53.

BERRY, S., BOLYCHEVTSEVA, Y. V., RÖGNER, M. & KARAPETYAN N.V. 2003. Photosynthetic and respiratory electron transport in the alkaliphilic cyanobacterium *Arthrospira (Spirulina) platensis*. *Photosynthesis Research,* 78**,** 67-76.

BINAGHI, L., DEL BORGHI, A., LODI, A., CONVERTI, A. & DEL BORGHI, M. 2003. Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. *Process Biochemistry,* 38**,** 1341-1346.

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

BOTHA, Z. 2004. SA set to become world's spirulina capital. *Engineering news.* Bedfordview, Creamer Medi's.

BOUSSIBA, S. & GIBSON, J. 1991. Ammonia translocation in cyanobacteria. *FEMS Microbiology Letters,* 88**,** 1-14.

BRIASSOULIS, D., PANAGAKIS, P., CHIONIDIS, M., TZENOS, D., LALOS, A., TSINOS, C., BERBERIDIS, K. & JACOBSEN, A. 2010. An experimental helical-tubular photobioreactor for continuous production of *Nannochloropsis* sp. *Bioresource Technology,* 101.

CAMACHO RUBIO, F., ACIE´N FERNANDEZ, F. G., SANCHEZ PE´ REZ, J. A., GARCıA CAMACHO, F. & MOLINA GRIMA, E. 1998. Prediction of Dissolved Oxygen and Carbon Dioxide Concentration Profiles in Tubular Photobioreactors for Microalgal Culture. *BIOTECHNOLOGY AND BIOENGINEERING,* 62**,** 71-86.

CARLSSON, A. S., VAN BEILEN, J. B., MÖLLER, R. & CLAYTON, D. 2007. Micro- and macro-algae: utility for industrial ??. Chippenham, University of York.

ÇELEKLI, A. & YAVUZATMACA, M. 2009. Predictive modeling of biomass production by *Spirulina platensis* as function of nitrate and NaCl concentrations. *Bioresource Technology,* 100**,** 1847-1851.

ÇELEKLI, A., YAVUZATMACA, M. & BOZKURT, H. 2009. Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Bioresource Technology,* 100**,** 3625-3629.

CHAUMONT, D. 1993. Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *Journal of Applied Phycology,* 5**,** 593-604.

CHENG, C.-G., HONG, Q.-H., LI, D.-T., FAN, M.-H. & CAI, X.-D. 2006a. Determination of trace elements in *Spirulina platensis* (Notdst.) Geitl. by flame atomic absorption spectrometry combined with microsampling pulse nebulization technique]. *Guang Pu Xue Yu Guang Pu Fen Xi = Guang Pu,* 26**,** 1735-1737.

CHENG, L., ZHANG, L., CHEN, H. & GAO, C. 2006b. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. *Separation and Purification Technology,* 50**,** 324-329.

CHOONAWALA, B. B. 2007. *Spirulina* Production in Brine Effluent from Cooling Towers. *Biotechnology.* Durban, DURBAN UNIVERSITY OF TECHNOLOGY.

CIFERRI, O. 1983. *Spirulina*, the Edible Microorganism. *American Society for Microbiology,* 47**,** 551-578.

COHEN, E. & ARAD, S. 1989. A closed system for outdoor cultivation of *Porphyridium*. *Biomass,* 18**,** 59-67.

COHEN, Z. & VONSHAK, A. 1991. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. *Phytochemistry,* 30**,** 205-206.

COHEN, Z., VONSHAK, A. & RICHMOND, A. 1987. Fatty acid composition of *Spirulina* strains grown under various environmental conditions. *Phytochemistry,* 26**,** 2255-2258.

COLLET, P., HÉLIAS, A., LARDON, L., RAS, M., GOY, R.-A. & STEYER, J.-P. 2010. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresource Technology,* 102**,** 207-214.

CONVERTI, A., LODI, A., DEL BORGHI, A. & SOLISIO, C. 2006a. Cultivation of *Spirulina platensis* in a combined airlift-tubular reactor system. *Biochemical Engineering Journal,* 32**,** 13-18.

CONVERTI, A., SCAPAZZONI, S., LODI, A. & CARVALHO, J. C. M. 2006b. Ammonium and urea removal by *Spirulina platensis*. *Journal of Industrial Microbiology & Biotechnology,* 33**,** 8-16.

DANESI, E. D. G., DE O. RANGEL-YAGUI, C., DE CARVALHO, J. C. M. & SATO, S. 2002. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy,* 23**,** 261-269.

DEMIRBAS, A. 2010. Use of algae as biofuel sources. *Energy Conversion and Management,* 51**,** 2738-2749.

DERNEKBASI, S., UNA, H., KARAYUCEL, I. & ARAL, O. 2010. Effect of Dietary Supplementation of Different Rates of Spirulina (*Spirulina platensis)* on Growth and Feed Conversion in Guppy (*Poecilia reticulata* Peters, 1860). *Journal of Animal and Veterinary Advances,* 9**,** 1395-1399.

DUAN, M., MA, W. X., LI, L. & SUN, X. T. 2001. [Determination of micro-elements in natural spirulina using FAAS]. *Guang Pu Xue Yu Guang Pu Fen Xi = Guang Pu,* 21**,** 868-870.

EWINGS, S. 2005. Growing algae.

FONTES, A. N. G., ANGELES VARGAS, M., MORENO, J., GUERRERO, M. G. & LOSADA, M. 1987. Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. *Biomass,* 13**,** 33-43.

GAL , Y. L. & ULBER, R. 2005. *Marine Biotechnology,* New York, Springer Berlin Heidelberg.

GALLAGHER, B. J. 2010. The economics of producing biodiesel from algae. *Renewable Energy,* 36**,** 158-162.

GEORGACAKIS, D., SIEVERS, D. M. & IANNOTTI, E. L. 1982. Buffer stability in manure digesters. *Agricultural Wastes,* 4**,** 427-441.

GONZÁLEZ-FERNÁNDEZ, C., MOLINUEVO-SALCES, B. & GARCÍA-GONZÁLEZ, M. C. 2010. Open and enclosed photobioreactors comparison in terms of organic matter utilization, biomass chemical profile and photosynthetic efficiency. *Ecological Engineering,* 36**,** 1497-1501.

GRIFFITHS, M. J., GARCIN, C., VAN HILLE, R. P. & HARRISON, S. T. L. 2011. Interference by pigment in the estimation of microalgal biomass concentration by optical density. *Journal of Microbiological Methods,* In Press, Uncorrected Proof.

GRIMA, E. M., FERN´ANDEZ, F. G. A. E., CAMACHO, F. G. & RUBIO, F. R. 2000. Scale-up of tubular photobioreactors. *Journal of Applied Phycology,* 12**,** 355–368.

GRIMA, E. M., PÉREZ, J. A. S., CAMACHO, F. G., SEVILLA, J. M. F. & FERNÁNDEZ, F. G. A. 1996. Productivity analysis of outdoor chemostat culture in tubular air-lift photobioreactors. *Journal of Applied Phycology,* 8**,** 369-380.

GROBBELAAR, J. U. 1982. Potential of algal production. 8**,** 79-85.

GROBBELAAR, J.U. 1989. Do light/dark cycles of medium frequency enhance phytoplankton productivity ? *Journal of Applied Phycology,* 1**,** 333-340.

GROBBELAAR, J. U. 1991. The influence of light/dark cycles in mixed algal cultures on their productivity. *Bioresource Technology,* 38**,** 189-194.

GROBBELAAR, J. U. 1994. Turbulence in mass algal cultures and the role of light/dark fluctuations. *Journal of Applied Phycology,* 6**,** 331-335.

GROBBELAAR, J. U. 2000. Physiological and technological considerations for optimising mass algal cultures. *Journal of Applied Phycology,* 12**,** 201–206.

GROBBELAAR, J. U. 2008. Factors governing algal growth in photobioreactors: the "open" versus "closed" debate. *Journal of Applied Phycology,* 21**,** 489-492.

GROBBELAAR, J. U., NEDBAL, L. & TICHÝ, V. 1996a. Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. *Journal of Applied Phycology,* 8**,** 335-343.

HARUN, R., SINGH, M., FORDE, G. M. & DANQUAH, M. K. 2009. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews,* 14**,** 1037-1047.

HSIEH, C.-H. & WU, W.-T. 2009. A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. *Biochemical Engineering Journal,* 46**,** 300-305.

JAISWAL, P., PRASANNA, R. & KASHYAP, A. K. 2005. Modulation of carbonic anhydrase activity in two nitrogen fixing cyanobacteria, *Nostoc calcicola* and *Anabaena* sp. *Journal of Plant Physiology,* 162**,** 1087-1094.

JANSSEN, M. 2002. Cultivation of microalgae: effect of light/dark cycles on biomass yield. Wageningen, Wageningen university.

JANSSEN, M., JANSSEN, M., DE WINTER, M., TRAMPER, J., MUR, L. R., SNEL, J. & WIJFFELS, R. H. 2000. Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light/dark cycles. *Journal of Biotechnology,* 78**,** 123-137.

JANSSEN, M., SLENDERS, P., TRAMPER, J., MUR, L. R. & WIJFFELS, R. 2001a. Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles. *Enzyme and Microbial Technology,* 29**,** 298-305.

JEAMTON, W. 1997. Molecular cloning and characterization of the phycocyanin genes from *Spirulina platensis* C I. *School of Bioresources and Technology.* King Mongkut's Institute of Technology Thonburi.

JIMÉNEZ, C., COSSÍO, B. R., LABELLA, D. & XAVIER NIELL, F. 2003a. The Feasibility of industrial production of *Spirulina (Arthrospira)* in Southern Spain. *Aquaculture,* 217**,** 179-190.

JIMÉNEZ, C., COSSÍO, B. R. & NIELL, F. X. 2003b. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. *Aquaculture,* 221**,** 331-345.

KARKOS, P. D., LEONG, S. C., KARKOS, C. D., SIVAJI, N. & ASSIMAKOPOULOS, D. A. 2008. *Spirulina* in Clinical Practice: Evidence-Based Human Applications. *eCAM***,** nen058-nen058.

KING, D. L. 1978. The role of carbon in eutrophication. *Water Pollution Control Federation,* 42**,** 2035-2051.

KOK, B. 1953. *Experiments on photosynthesis by Chlorella in flashing light.,* Washington D.C, Carnegie Institution of Washington.

KULSHRESHTHA, A., ZACHARIA, A. J., JAROULIYA, U., BHADAURIYA, P., PRASAD, G. B. K. S. & BISEN, P. S. 2008. *Spirulina* in health care management. *Current Pharmaceutical Biotechnology,* 9**,** 400-405.

LAORAWAT, S. 1995. Effects of Environmental Factors on Photosynthesis and Productivity of *Spirulina platensis* in Outdoor Cultures. *School of Bioresources and Technology.* King Mongkut's Institute of Technology Thonburi.

LEE, Y. K. 2001. Microalgal mass culture systems and methods: Their limitation and potential *Journal of Applied Phycology,* 13**,** 307–315.

LEEMA, J. T., KIRUBAGARAN, R., VINITHKUMAR, N. V., DHEENAN, P. S. & KARTHIKAYULU, S. 2010. High value pigment production from *Arthrospira (Spirulina) platensis* cultured in seawater. *Bioresource Technology,* 101**,** 9221-9227.

LIU, Y., LI, G., GUO, F., JIANG, W., LI, Y. & LI, L. 2010. Large-scale production of magnetosomes by chemostat culture of *Magnetospirillum gryphiswaldense* at high cell density. *Microbial Cell Factories,* 9**,** 99-99.

MA, Z. & GAO, K. 2009. Spiral breakage and photoinhibition of *Arthrospira platensis* (Cyanophyta) caused by accumulation of reactive oxygen species under solar radiation. *Environmental and Experimental Botany,* 68**,** 208-213.

MADHYASTHA, H. K. & VATSALA, T. M. 2007. Pigment production in *Spirulina fussiformis* in different photophysical conditions. *Biomolecular Engineering,* 24**,** 301-305.

MARKOU, G. & GEORGAKAKIS, D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy,* xxx**,** xxx–xxx.

MASOJÍDEK, J., TORZILLO, G., SVEN ERIK, J. & BRIAN, F. 2008. Mass Cultivation of Freshwater Microalgae. *Encyclopedia of Ecology.* Oxford, Academic Press.

MAZO, V. K., GMOSHINSKIĬ, I. V. & ZILOVA, I. S. 2004. [Microalgae *Spirulina* in human nutrition]. *Voprosy Pitaniia,* 73**,** 45-53.

MCCONNAUGHEY, T. A. & WHELAN, J. F. 1997. Calcification generates protons for nutrient and bicarbonate uptake. *Earth-Science Reviews,* 42**,** 95-117.

MCDONALD, M. S. 2003. *Photobiology of higher plants*, John Wiley and Sons.

MENDIOLA, J. A., GARCÍA-MARTÍNEZ, D., RUPÉREZ, F. J., MARTÍN-ÁLVAREZ, P. J., REGLERO, G., CIFUENTES, A., BARBAS, C., IBAÑEZ, E. & SEÑORÁNS, F. J. 2008. Enrichment of vitamin E from *Spirulina platensis* microalga by SFE. *The Journal of Supercritical Fluids,* 43**,** 484-489.

MERZ, M. U. E. 1992. The biology of carbonate precipitation by cyanobacteria. *Facies,* 26**,** 81-101.

MIYAMOTO, K., WABLE, O. & BENEMANN, J. R. 1988. Vertical tubular reactor for microalgae cultivation. *Biotechnology Letters,* 10**,** 703-708.

MORAIS, M. G., RADMANN, E. M., ANDRADE, M. R., TEIXEIRA, G. G., BRUSCH, L. R. F. & COSTA, J. A. V. 2009. Pilot scale semicontinuous production of *Spirulina* biomass in southern Brazil. *Aquaculture,* 294**,** 60-64.

MORENO-GARRIDO, I. 2008. Microalgae immobilization: Current techniques and uses. *Bioresource Technology,* 99**,** 3949-3964.

NANDEESHA, M. C., GANGADHARA, B., MANISSERY, J. K. & VENKATARAMAN, L. V. 2001. Growth performance of two Indian major carps, catla (Catla catla) and rohu (Labeo rohita) fed diets containing different levels of *Spirulina platensis*. *Bioresource Technology,* 80**,** 117-120.

OGBONNA, J. C. & TANAKA, H. 2000. Light requirement and photosynthetic cell cultivation – Development of processes for efficient light utilization in photobioreactors. *Journal of Applied Phycology,* 12**,** 207–218.

OHMORI, M., OHMORI, K. & STROTMANN, H. 1977. Inhibition of nitrate uptake by ammonia in a blue-green alga, *Anabaena cylindrica*. *Archives of Microbiology,* 114**,** 225-229.

OLIVEIRA, M. A. C. L. D., MONTEIRO, M. P. C., ROBBS, P. G. & LEITE, S. G. F. 1999. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensi*s biomass at different temperatures. *Aquaculture International,* 7**,** 261-275.

ONCEL, S. S. & AKPOLAT, O. 2006. An integrated photobioreactor system for the production of *Spirulina platensis*. *Biotechnology* 5**,** 365-372.

PANDEY, J. P., PATHAK, N. & TIWARI, A. 2010a. Standardization of pH and Light instensity for the Biomass Production of *Spirulina platensis*. *Algal Biomass,* 1**,** 93-102.

PANDEY, J. P. & TIWARI, A. 2010. Optimisation of BiomassProduction of *Spirulina maxima*. *Algal Biomass,* 1**,** 20-32.

PANDEY, J. P., TIWARI, A. & MISHRA, R. M. 2010b. Evaluation of Biomass Production of *Spirulina maxima* on different Reported Media. *Algal Biomass,* 1**,** 70-81.

PAP´AˇCEK, I. S. 2005. Photobioreactors for cultivation of microalgae under strong irradiances: Modelling, simulation and design. *Faculty of Mechatronics and Interdisciplinary Engineering Studies.* Liberec, Technical University of Liberec.

PAREDES-CARBAJAL, M. C., TORRES-DURÁN, P. V., DÍAZ-ZAGOYA, J. C., MASCHER, D. & JUÁREZ-OROPEZA, M. A. 1997. Effects of dietary *Spirulina maxima* on endothelium dependent vasomotor responses of rat aortic rings. *Life Sciences,* 61**,** PL211-PL219-PL211-PL219.

PELCZAR, M. J., CHAN, E. C. S. & KRIEG, N. R. 2010. *Microbiology: An Application Based Approach,* New Delhi, Tata MaGraw Hill Education Private Limited.

PELIZER, L. H., DANESI, E. D. G., RANGEL, C. D. O., SASSANO, C. E. N., CARVALHO, J. C. M., SATO, S. & MORAES, I. O. 2003. Influence of inoculum age and concentration in *Spirulina platensis* cultivation. *Journal of Food Engineering,* 56**,** 371-375.

PHAM QUOC, K. & DUBACQ, J.-P. 1997. Effect of growth temperature on the biosynthesis of eukaryotic lipid molecular species by the cyanobacterium *Spirulina platensis*. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism,* 1346**,** 237-246.

PIORRECK, M., BAASCH, K.-H. & POHL, P. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry,* 23**,** 207-216.

PULZ, O. 2001. Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology,* 57**,** 287-293.

QIANG, H., ZARMI, Y. & RICHMOND, A. 1998. Combined effects of light intensity, light-path and culture density on output rate of *Spirulina platensis* (Cyanobacteria). *European Journal of Phycology,* 33**,** 165-171.

RADMER, R. J. 1996. Algal Diversity and Commercial Algal Products (New and valuable products from diverse algae may soon increase the already large market for algal products). *American Institute of Biological Science,* 46**,** 263-270.

RAFIGUL, I. M., JALAL, K. C. A. & ALAM, M. 2005. Environmental Factors for Optimisation of *Spirulina* Biomass in Laboratory Culture. *Biotechnology and Bioengineering,* 4**,** 19-22.

RAFIQUL ISLAM, M. D., HASSAN, A., SULEBELE, G., OROSCO, C. & ROUSTAIAN, P. 2003. Influence of Temperature on Growth and Biochemical Composition of *Spirulina platensis* and *S. fusiformis*. *Iranian Int. J. Sci,* 4**,** 97-106.

REUTER, W. & MÃLLER, C. 1993. New trends in photobiology: Adaptation of the photosynthetic apparatus of cyanobacteria to light and co2. *Journal of Photochemistry and Photobiology B: Biology,* 21**,** 3-27.

RICHMOND, A. 2004. *Handbook of Microalgal Culture: Biotechnology and Applied Phycology,* Ames, Blackwell Science Ltd.

RIDING, R. 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoicâ€“Cambrian changes in atmospheric composition. *Geobiology,* 4**,** 299-316.

RODRIGUES, M. S., FERREIRA, L. S., CONVERTI, A., SATO, S. & CARVALHO, J. C. M. 2010.Fed-batch cultivation of *Arthrospira (Spirulina) platensis*: Potassium nitrate and ammonium chloride as simultaneous nitrogen sources. *Bioresource Technology,* 101**,** 4491-4498.

ROSELLO SASTRE, R., CSÖGÖR, Z., PERNER-NOCHTA, I., FLECK-SCHNEIDER, P. & POSTEN, C. 2007. Scale-down of microalgae cultivations in tubular photo-bioreactors--A conceptual approach. *Journal of Biotechnology,* 132**,** 127-133.

RUSCH, K. 2003. The hydraulically integrated serial turbidostat algal reactor (HISTAR) for microalgal production. *Aquacultural Engineering,* 27**,** 249-264.

SAJILATA, M. G., SINGHAL, R. S. & KAMAT, M. Y. 2008. Fractionation of lipids and purification of [gamma]-linolenic acid (GLA) from *Spirulina platensis*. *Food Chemistry,* 109**,** 580-586.

SÁNCHEZ, M., BERNAL-CASTILLO, J., ROZO, C. & RODRÍGUEZ, I. 2003. *Spirulina (arthrospira*): an edible microorganism. A review. 7**,** 43-48.

SASSANO, C. E. N., GIOIELLI, L. A., FERREIRA, L. S., RODRIGUES, M. S., SATO, S., CONVERTI, A. & CARVALHO, J. C. M. 2010. Evaluation of the composition of continuously-cultivated *Arthrospira (Spirulina) platensis* using ammonium chloride as nitrogen source. *Biomass and Bioenergy,* 34**,** 1732-1738.

SASTRE, R., ROSELLO. , CSÖGÖR, Z., PERNER-NOCHTA, I., PASCALE, F.-S. & POSTEN, C. 2007. Scale-down of microalgae cultivations in tubular photo-bioreactors--A conceptual approach. *Journal of Biotechnology,* 132**,** 127-133.

SEALE, D. B., BORAAS, M. E. & WARREN, G. J. 1987. Effects of sodium and phosphate on growth of cyanobacteria. 21**,** 625-632.

SIGEE, D. C. 2005. *Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment*, John Wiley and Sons.

SORGELOOS, P., VAN OUTRYVE, E., PERSOONE, G. & ATTOIR-REYNAERTS, A. 1975. New Type of Turbidostat with Intermittent Determination of Cell Density Outside the Culture Vessel. *Applied and Environmental Microbiology,* 31**,** 327-331.

SOROKIN, C. & KRAUSS, R. W. 1965. The dependent of cell division *Chlorella* on the temperature and light intensity. *Amer. Jour. Bot,* 52**,** 331-339.

SOUTH, G. R. & WHITTICK, A. 1987. *Introduction to phycology,* London, Blackwell scientific publication.

STEVENSON, R. J., BOTHWELL, M. L. & LOWE, R. L. 1996. *Algal ecology: freshwater benthic ecosystems*, Academic Press.

TERRY, K. L. 1986. Photosynthesis in modulated light: Quantitative dependence of photosynthetic enhancement on flashing rate. *Biotechnology and Bioengineering,* 28**,** 988-995.

THAMMATHORN, S. 2001 Factors affecting coiling and uncoiling of *Spirulina platensis* C1. *School of bioresources and technology.* King Mongkut's University of Technology Thonburi.

TOLGA, G. K., AYßEG, L. Z. & ÜLKNUR, A. 2006. The Growth of *Spirulina platensis* in Different Culture Systems

Under Greenhouse Condition. *Turk J Biol,* 31**,** 47-52.

TORZILLO, G., CARLOZZI, P., PUSHPARAJ, B., MONTAINI, E. & MATERASSI, E. 1993. A two-plane tubular photobioreactor for outdoor culture of *Spirulina*. *Biotechnology and Bioprocess Engineering*, 42, 891-898.

TORZILLO, G., PUSHPARAJ, B., MASOJIDEK, J. & VONSHAK, A. 2003. Biological constraints in algal biotechnology. *Biotechnology and Bioprocess Engineering,* 8**,** 338-348.

UGWU, C. U. & AOYAGI, H. 2008. Influence of shading inclined tubular photobioreactor surfaces on biomass productivity of *C. sorokiniana*. *Photosynthetica,* 46**,** 283-285.

UGWU, C. U., AOYAGI, H. & UCHIYAMA, H. 2007. Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *PHOTOSYNTHETICA,* 45.

UGWU, C. U., AOYAGI, H. & UCHIYAMA, H. 2008. Photobioreactors for mass cultivation of algae. *Bioresource Technology,* 99**,** 4021-4028.

USLU, L. H., ISIK, O., KOC, K. & GOKSAN, T. 2011. The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *African Journal of Biotechnology,* 10**,** 386-389.

USLU, L. H., ISIK, O., SAYIN, S., DURMAZ, Y., GOKSAN, T. & GOKPINAR, S. 2009. The effect of Temperature on Protein and Amino Acid Composition of *Spirulina platensis*. *E.U. Journal of Fisheries & Aquatic Sciences,* 26**,** 139-142.

VAN VOOREN, L., LESSARD, P., OTTOY, J. P. & VANROLLEGHEM, P. A. 1999. pH Buffer Capacity Based Monitoring of Algal Wastewater Treatment. *Environmental Technology,* 20**,** 547-561.

VIEIRA COSTA, J. A., COZZA, K. L., OLIVEIRA, L. & MAGAGNIN, G. 2001. Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments. *World Journal of Microbiology and Biotechnology,* 17**,** 439-442.

VIEIRA COSTA, J. A., LUCIANE MARIA COLLA & FILHO, A. P. D. 2002. *Spirulina platensis* Growth in Open Raceway Ponds Using Fresh Water Supplemented with Carbon, Nitrogen and Metal Ions. 58c**,** 76-80.

VONSHAK, A. & RICHMOND, A. (1988) Mass production of the blue-green alga Spirulina: An overview. *Biomass,* 15**,** 233-247.

WILLEY, J. M., SHERWOOD, L. M. & WOOLVERTON, J. C. 2008. *Microbiology (seventh Edition),* New York, McGraw-Hill.

XUE, S., SU, Z. & CONG, W. 2010. Growth of *Spirulina platensis* enhanced under intermittent illumination. *Journal of Biotechnology,* In Press, Uncorrected Proof.

ZARETSKAIA, E. S., GMOSHINSKIĬ, I. V., MAZO, V. K., ZORIN, S. N. & ALESHKO-OZHEVSKIĬ, I. P. 2004. [Essential trace elements distribution in food micro algae *Spirulina platensis* biomass fractions]. *Voprosy Pitaniia,* 73**,** 28-31.

APPENDIX A

Schlösser *Spirulina* medium

The medium is made up of two components, A and B, which are autoclaved apart. Once the components have cooled, they are mixed in equal proportion to make up the final medium. The PIV and Chu metal solutions and Vitamin B12 are added afterwards. Finally the medium is inoculated with Spirulina.

Solution A

Solution A is made up of dissolving 27.22 g/l $NaHCO_3$, 8.06 Na_2CO_3, and 1.00 g/l K_2HPO_4 to 2 liter milli-Q water in a pyrex schott bottle and autoclaved at 121 ^{O}C at 1 bar for 15 min.

Solution B

Solution B is made up by dissolving 5.00g/l $NaNO_3$, 2.00 K_2SO_4, 2.00 NaCl, 0.40 g $MgSO_4.7H_2O$ and 0.08 g $CaCl_2.2H_2O$ to 2 liter milli-Q water in a pyrex schott bottle and autoclaved at 121 ^{O}C at 1 bar for 15 min.

PIV solution

PIV solution is made up by dissolving 1.5 g Na_2EDTA, 194 mg $FeCl_3.6H_20$, 82 mg $MnCl_2.4H_20$, 10 mg $ZnCl_2$, 4 mg $CoCl_2.6H_2O$, 8 mg $Na_2MoO4.2H_2O$ to 2 liter milli-Q water in a pyrex schott bottle and autoclaved at 121 ^{O}C at 1 bar for 15 min.

Chu

Chu solution is made up by dissolving 100 mg Na_2EDTA, 1.236 mg H_3BO_3, 39 mg $CuSO_4.5H_2O$, 88 mg $ZnSO_4.7H_2O$, 40 mg $CoCl_2.6H_2O$, 25 mg $MnCl_2.4H_2O$, 25 mg $Na_2MoO_4.2H_2O$ to 2 liter milli-Q water in a pyrex schott bottle and autoclaved at 121 ^{O}C at 1 bar for 15 min.

Vitamin B12

Vitamin B12 for injection made up to 1 mg/ml is used.

Flask make-up

The culture is made up to 100 ml in 250 ml pre- autoclaved and sealed conical flasks. 50ml of solution A and B are added to each flask, followed by 0.6 ml PIV and 0.1 ml Chu solutions. Finally, 0.015 ml vitamin B12 solution is added. Once the solution have been mixed, each flask is inoculated with 10 ml of its respective inoculum and incubated in the growth room on a shaker table.

APPENDIX B

Provisional patent

The novel tubular photobioreactor's provisional patent approval published on the patent journal 2010. The patent number was 2010/05610 and it's the last one on this patent journal page.

2010/05596 ~ Complete ~ 54: MONITORING OF CONVERGENCE OF EVACUATION SURFACES ~ 71:1. CSIR ~ 72:1. BILGERI REINHARD JOSEF~ 33: South Africa ~ 31: 2008/06252 ~ 32: 18/07/2008.

2010/05597 ~ Provisional ~ 54: SYSTEM FOR IDENTIFYING A RECIPIENT AND MAKING A PAYMENT ~ 71:1. RABIE MELCHIOR ~ 72:1. Rabie MELCHIOR~ 33: South Africa ~ 31: 2008/06252 ~ 32: 18/07/2008.

2010/05598 ~ Complete ~ 54: HEAT TREATED GALVANNEALED STEEL MATERIAL AND A METHOD FOR ITS MANUFACTURE ~ 71:1. SUMITOMO METAL INDUSTRIES LTD ~ 72:1. TAMOTSU TOKI 2. ATSUSHI TOMIZAWA 3. NOBUSATO KOJIMA 4. KAZUYA ISHII 5. KAZUHITO IMAI 6. TOYOMITSU NAKAMURA 7. NAOKI SHIMADA~ 33: Japan ~ 31: 2008-016531 ~ 32: 28/01/2008.

2010/05599 ~ Complete ~ 54: COVER AND METHOD FOR THE PRODUCTION THEREOF ~ 71:1. AMCOR FLEXINLES KREUZLINGEN LTD ~ 72:1. WOLTERS DETLEF 2. LOEBELT ROLAND~ 33: European Patent Office (EPO) ~ 31: 08405031.9 ~ 32: 05/02/2008.

2010/05600 ~ Complete ~ 54: AMPK MODULATORS ~ 71:1. MERCURY THERAPEUTICS INC ~ 72:1. BIRNBERG NEAL C 2. LIU HONG 3. WENG QING PENG 4. SHANG HAIBO 5. YIN PAN 6. RAJUR SHARANAPPA B 7. KIM HWA-OK 8. SALGAONKAR PARESH D 9. PEET NORTON P~ 33: United States of America ~ 31: 61/051,200 ~ 32: 07/05/2008.

2010/05602 ~ Complete ~ 54: IMIDAZO [1,2-B]PYRIDAZINE DERIVATIVES FOR THE TREATMENT OF C-MET TYROSINE KINASE MEDIATED DISEASE ~ 71:1. NOVARTIS AG ~ 72:1. FURET PASCAL 2. MCCARTHY CLIVE 3. SCHOEPFER JOSEPH 4. SPANKA CARSTEN 5. STANG MELANIE 6. STAUFFER FREDERIC~ 33: European Patent Office (EPO) ~ 31: 08152068.6 ~ 32: 28/02/2008.

2010/05603 ~ Complete ~ 54: BRIDGED,BICYCLIC HETEROCYCLIC OR SPIRO BICYCLIC HETEROCYCLIC DERIVATIVES OF PYRAZOLO [1,5-A]PYRIMIDINES,METHODS FOR PREPARATION AND USES THEREOF ~ 71:1. WYETH LLC ~ 72:1. LEVIN JEREMY IAN 2. HOPPER DARRIN WILLIAM 3. TORRES NANCY 4. DUTIA MINU DHANJISH 5. BERGER DAN MAARTEN 6. WANG XIAOLUN 7. DI GRANDI MARTIN JOSEPH 8. ZHANG CHUNCHUN 9. DUNNICK ALEJANDRO LEE~ 33: United States of America ~ 31: 61/116,809 ~ 32: 21/11/2008.

2010/05604 ~ Complete ~ 54: PROCESS FOR THE PRODUCTION OF BIOLOGICALLY ATIVE HIV-1 TAT PROTEIN ~ 71:1. UNIVERSITA DEGLI STUDI DI URBINO 2. ISTITUTO SUPERIORE DI SANITA ~ 72:1. ENSOLI BARBARA 2. MAGNANI MAURO~ 33: United Kingdom ~ 31: 0802224.6 ~ 32: 06/02/2008.

2010/05605 ~ Complete ~ 54: HYPOCHLORITE BASED BIOCIDE COMBINATION FOR USE IN AGRICULTURAL APPLICATIONS ~ 71:1. INDUSTRIE DE NORA S P A ~ 72:1. FAITA GIUSEPPE~ 33: Italy ~ 31: MI 2008 A 000027 ~ 32: 09/01/2008.

2010/05606 ~ Complete ~ 54: DEVICE ON A COAL CHARGING CAR FOR LIFTING COVERS FROM FILLING HOLE FRAMES IN THE FURNACE ROOF OF A COKE FURNACE AND FOR CLEANING THE FILLING HOLE FRAMES ~ 71:1. UHDE GMBH ~ 72:1. SCHUCKER FRANZ-JOSEF 2. KNOCH RALF~ 33: Germany ~ 31: 10 2008 008 291.0 ~ 32: 07/02/2008.

2010/05607 ~ Complete ~ 54: SHUTTER HAVING AN INFLATABLE PERIPHERAL SEAL,AND SEALING SYSTEM COMPRISING IT FOR A MULTIPLE-CHAMBER FURNACE PORT ~ 71:1. SOLIOS CARBONE ~ 72:1. BENHARBON GERARD 2. MAHIEU PIERRE~ 33: France ~ 31: 0850921 ~ 32: 13/02/2008.

2010/05608 ~ Complete ~ 54: PHARMACEUTICAL COMPOSITIONS OF ENTACAPONE CO-MICRONIZED WITH SUGAR ALCOHOLS ~ 71:1. WOCKHARDT RESEARCH CENTRE ~ 72:1. KAPOOR RITESH 2. MATE SANJAY 3. TALWAR MUNISH 4. JAIN GIRISH KUMAR~ 33: India ~ 31: 264/MUM/2008 ~ 32: 06/02/2008.

2010/05609 ~ Provisional ~ 54: AN APPARATUS FOR THE DISABLED,ELDERLY OR INCAPACITATED ~ 71:1. VAAL UNIVERSITY OF TECHNOLOGY ~ 72:1. VAN GRIJP REYNARD~ 33: India ~ 31: 264/MUM/2008 ~ 32: 06/02/2008.

2010/05610 ~ Provisional ~ 54: A NOVEL TUBULAR PHOTO BIOREACTOR ~ 71:1. VAAL UNIVERSITY OF TECHNOLOGY ~ 72:1. MAKONDE KUTAMA 2. STEGMANN PETER~ 33: India ~ 31: 264/MUM/2008 ~ 32: 06/02/2008.

APPENDIX C

International patent

The international patent published on the world intellectual property website.

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2012019206) A TUBULAR PHOTO BIOREACTOR

PCT Biblio. Data | Description | Claims | National Phase | Notices | Documents

Latest bibliographic data on file with the International Bureau ⇨ Submit observation

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Priority Data: 2010/05610 05.08.2010 ZA

Title **(EN)** A TUBULAR PHOTO BIOREACTOR
(FR) PHOTOBIORÉACTEUR TUBULAIRE

Abstract: **(EN)**This invention relates to a bioreactor, more particularly to a tubular photo bioreactor (TPB), which is capable of stimulating plant growth, especially micro algal growth. The tubular photo bioreactor includes a reservoir for holding a liquid containing plant material, wherein the reservoir is devoid from light, a pump in fluid communication with the reservoir and a length of transparent tube extending from the pump to the reservoir through which the liquid can be circulated. The tubular photo bioreactor further includes a frame adapted to support the tube extending between the pump and the