

Figure.10 The variation of the heat transfer enhancement factor for various continuous frequencies (a) $q'' = 85 \text{ W/m}^2$ (b) $q'' = 946.017 \text{ W/m}^2$.

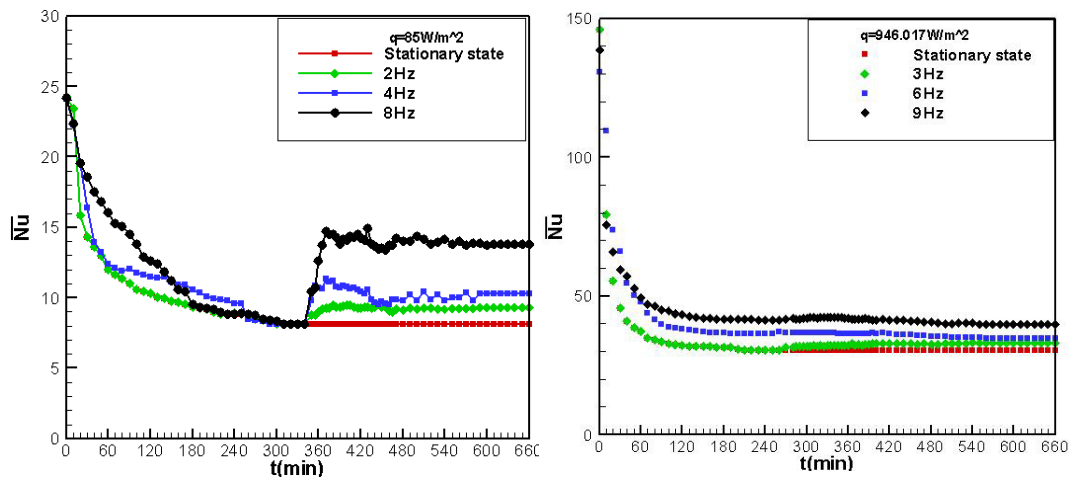


Figure. 11 The comparisons of variations the time- average Nusselt number for of Interrupted frequencies with the stationary state (a) $q'' = 85 \text{ W/m}^2$ (b) $q'' = 946.017 \text{ W/m}^2$.

The Growth Resources Enhancing Lipids for Bio-diesel

Production in *Chlamydomonas reinhardtii*.

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Abstract

The growth of the microalgae can be enhanced by optimizing the resources which will boost its growth rate and lipid yield. These resources include growth conditions and nutrient concentration and availability. This study sought to determine the effect of the growth resources of light, photoperiod, carbon dioxide and urea concentration and nitrogen limitation on growth rate and lipid production in *Chlamydomonas reinhardtii*. Screening and isolation of the alga was carried out from samples collected from the Nairobi Dam and grown in Modified Bold's Basal Media. Various isolates of algae were collected and *C. reinhardtii* was chosen as the candidate species. The results indicated an optimum growth at light intensity of $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ in an 18-hour photoperiod. The yield data on biomass of the alga grown at this photoperiod had a mean dry weight of $53.5 \pm 1.29\text{g}$ after a 3 week growth period while a 6-hour photoperiod recorded $25 \pm 1.09\text{g}$ translating to daily increase of 2.5g/day and 1.2g/day respectively. *Chlamydomonas reinhardtii* showed a tolerance response of 15% CO_2 concentration and a robust growth in urea of 2.4g/l , giving lipid yield of 4.9ml/g of the dry weight. Growth in 1.2g/l of urea also gave a yield of 5ml/g of dry weight. The alga grown in 100gms of nitrogen gave a lipid yield of 0.592g of in contrast to 0.102g for those grown in 250gms . These results showed that by optimizing these growth resources a higher biomass density and lipid yield for bio-diesel production can be obtained from this alga.

Keywords: *Chlamydomonas reinhardtii*, lipids, growth resources.

1. Introduction

The need for alternative sources of energy has provided many ways to produce electricity, such as energy from the wind, hydroelectricity, and solar. To meet the world's transport needs, fuels which produce high amount of energy such as petrol, diesel or kerosene are needed. These fuels are all obtained by refining petroleum, whose dependency has a lot of drawbacks, major of which is the deterioration of the environment as a result of accumulation of CO_2 and NO_x gases.

The demand for energy is continually increasing and to protect the environment and maintain a sufficient energy for the supply, a concerted effort is being made to advance renewable energy alternatives which include the bio-fuels.

In order to maintain a sufficient energy supply while at the same time protecting the environment, efforts are being made to advance the renewable energy alternatives such as the bio-fuels. An attractive feedstock for bio-

fuel production should contain high amount of oil, have least impact on the environment and have low cost of production. Most crops being used currently such as *Jatropha*, soy bean, and sunflower, do not meet these requirements (Schubert, 2006) and this has ignited interest in microalgae as the most viable feedstock. Compared to these crops, microalgae can produce up to 30 times more oil per unit of growth area than land plants (Haag 2007) and can achieve a photosynthetic efficiency of 11.6% whereas most plants have a rate of 1-2% (Vasudevan and Briggs, 2008) resulting in high biomass and lipid content. They therefore have the most potential as a bio-diesel feedstock only if the technical challenges in culturing and optimization of lipid yield can be overcome.

2. Literature review

Lipid content of algae is an important parameter that determines the economy of algae bio-diesel production (Chisti, 2008). Depending on the species, microalgae contain a variety of different lipids, hydrocarbons and other complex oils. It has been observed that algae produce enormous amount of lipids when put into stressful environments such as nutrient deprivation, (Piorreck *et al.*, 1984). For instance, a strain of algae put into a nutrient deficient environment can go from 22% to 58% oil content per dry mass (Sheehan *et al.*, 1998) but some strains can reach as high as 80% (Metting, 1996; Spolaore *et al.*, 2006). One way to increase lipid content of microalgae is to manipulate the growth nutrient of algae. Nitrogen source and concentration in the growth media greatly influence algae lipid yield (Shen *et al.*, 2009). High biomass and high lipid yield have been achieved when heterotrophic algae are placed in low light and supplied organic carbon rather than carbon dioxide as carbon source (Miao and Wu, 2004; Xu *et al.*, 2006). Miao and Wu (2004) found autotrophic *Chlorella protothecoides* possessed a lipid content of 14.57% compared to 55.2% when grown heterotrophically. Xu *et al.* (2006) also reported the lipid content of the *Chlorella protothecoides* and showed an exponential growth in heterotrophic conditions.

Light is an important factor in defining optimal conditions for the algal culture (Falkowski *et al.*, 1985) and the efficiency of microalgal culture is controlled by the intensity of light and photoperiod. The carbon dioxide as a carbon source is very important in algal growth and is the sole source of carbon for the process of photosynthesis and biomass production.

Thus, owing to fast growth and high lipid content when subjected to optimum growth resources, microalgae are considered the best feedstock for the production of bio-fuels. In order to develop a cost-effective second generation feedstock for bio-fuel production, there is still the need to improve both the growth rate and lipid content of the microalgal strains such as optimization of the culture conditions. Faster growth rates of the microalgae can be achieved by manipulations of the environmental conditions and nutrient concentration and availability (Converti *et al.*, 2009; Rudolfi *et al.*, 2009).

3. Material and methods

The *Chlamydomonas reinhardtii* was isolated from water samples collected from the Nairobi Dam and the pure culture grown in the Modified Bold's Basal Media. The cultures were maintained at temperatures between 20°C and 25°C with a pH range of 6.5-7.5.

The effect of light intensity was studied by growing four replicates of the alga in increasing light intensities from 60 to 647 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Different light intensities were obtained by varying the distance between the cultures and

light source. Illumination intensities up to $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ were provided by fluorescent lamps whereas those above $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ were from bulbs. The test tubes were fitted with cotton plugs, through which bubbling tubes were passed to supply the cultures with a 0.03 % CO_2 . After inoculation the cultures were placed in a 25°C constant temperature water bath. A colorimeter was used to measure the daily changes in optical density whereby in the logarithmic growth phase cells number was related to optical density.

Growth rate, k , was calculated from the integrated growth equation:

$$K = \log_2 \frac{O.D._1}{O.D._0} \times \frac{1}{t}$$

Where, $O.D._0$ and $O.D._1$ are optical densities at the beginning and end of the time interval, t . By using logarithms to the base 2, and the unit of time as one day, the growth constant, k , becomes equivalent to the number of doublings per day

The effect of the photoperiod was studied by growing the alga in different periods of illumination time of 0, 6, 12 and 18 hours. Sixteen flasks were used for growing the alga. To allow for varying the photoperiod, four were labeled '6' and exposed to light for six hours, four were labeled '12' and the exposure time was twelve hours and the other four were labeled '18' and exposed to light for eighteen hours. The final four were labeled 'Dark' and were grown without light. Frequent transfers to fresh medium were required to prevent a reduction in growth rate before the lag phase was passed. The intensity of the illumination for all the replicates was kept at $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ and fed with CO_2 concentration of 0.03%. The growth rate was assayed by taking cell counts daily using a hemacytometer for all the durations of illumination. Harvesting was done at the end of the exponential phase and the mean weights compared.

To study the effect of carbon dioxide concentration on growth of the alga sixteen identical flasks were set up and divided into four groups and each group aerated with different composition of inlet carbon dioxide of concentrations 0.03, 5, 10 and 15%. Each group was then fitted with two 40 W fluorescent lights set up to give light intensity of $240 \mu\text{mol m}^{-2} \text{s}^{-1}$. A colorimeter was used to measure the optical density of the cultures from which the growth rate was calculated.

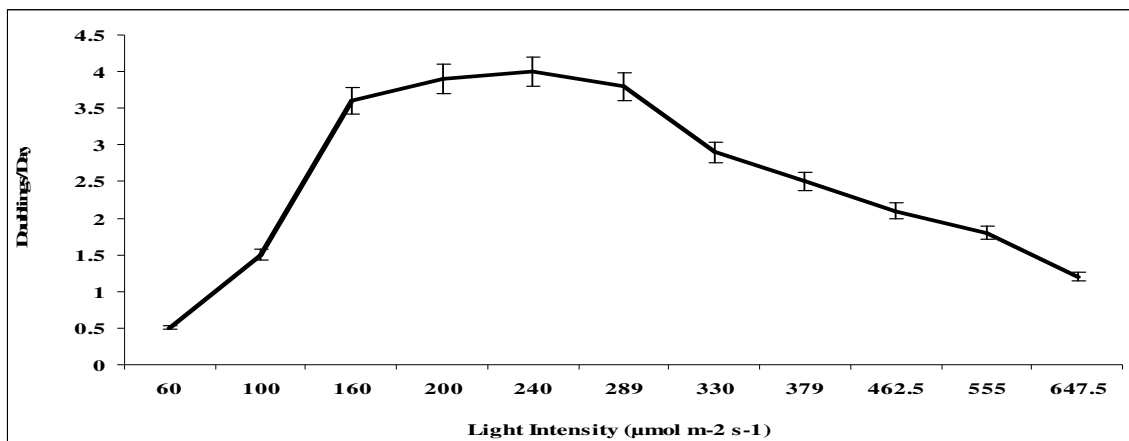
The source of nitrogen for the alga was urea. To assay its effect of on growth, twelve 1000 ml beakers were used for growing the alga each with three different concentrations of urea. The effect of urea on growth rate was studied by carrying out daily cell counts from each of the beakers and the mean cell counts recorded. At stationary phase the alga was harvested and mean dry weights compared. Lipids were also extracted and yield compared.

4. Results

The intensity of light showed a remarkable effect on the growth of *C. reinhardtii*. Figure 1 below shows the mean doublings per day for the alga grown under varying light intensities with all other environmental conditions kept constant. There was a highly significant difference ($df=10$, $F=178.467$, $p<0.05$) in growth rate of the alga under different light intensities.

From the results, the growth rate for *C. reinhardtii* grown in light intensity of $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ produced the highest growth. Growth above and below this range was diminished.

Figure 1: Effect of light intensity on growth rate of *Chlamydomonas reinhardtii*. Error bars represent standard deviation.



The 18-hour photoperiod showed the fastest growth rate attaining a high stationary phase cell yield of 3.8294×10^6 cells/ml, whereas 6 hour illumination period showed the least growth rate and reached the lowest stationary phase cell yield of 9.0×10^5 cells/ml. Those grown for 12 hours attained a yield of 2.1879×10^6 cells/ml at the stationary phase. With no other source of carbon provided, *C. reinhardtii* grown in the dark showed the least growth. The ANOVA output showed a highly significant difference ($F=25.213$, $df=3$, $p<0.05$) in cell counts for *C. reinhardtii* grown under different lengths of illumination time. Although the cultures were inoculated with the same biomass density, the stationary phase biomass yield varied considerably for the various treatments applied.

Figure 2 below illustrates the mean dry weights of the alga grown under different durations of exposure to light. It is seen from the figure that those exposed to 18 hours of light had the highest biomass yield compared to 12-hour and 6-hour exposure. The figure shows a progressive increase in biomass yield as the duration of illumination was increased. In absence of light, there was very minimal growth as shown in the figure.

Figure 2: Comparison of the mean dry weights of algae grown in 0, 6, 12 and 18 hours of illumination. Error bars represent standard deviation.

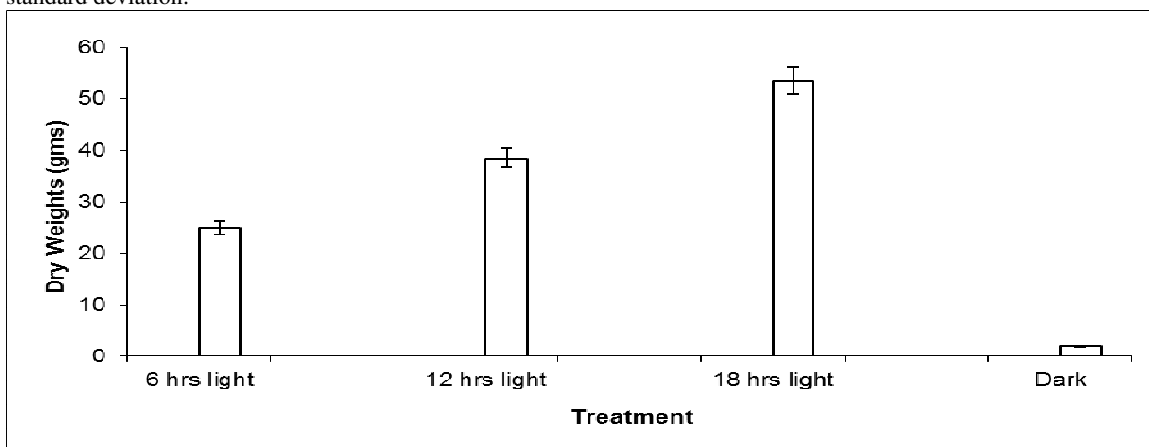
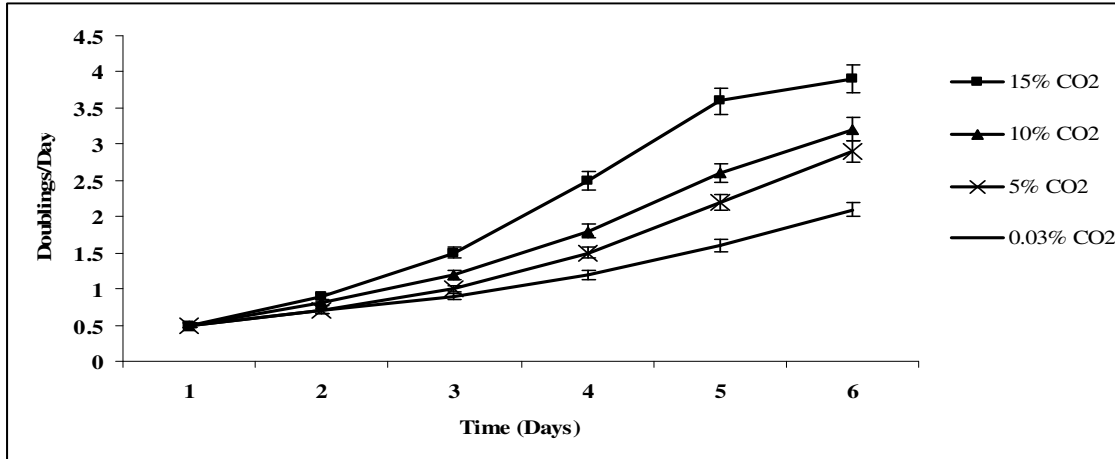


Figure 3 shows the doublings per day for *C. reinhardtii* grown in carbon dioxide of concentrations ranging from 0.03% to 15%. There was a highly significant difference in the growth rates of the alga under different concentrations of carbon dioxide ($df=3$, $F=4.492$, $p<0.05$).

From the results it was noted that *C. reinhardtii* grown in 15% CO₂ had the highest growth rate compared to 10%, 5% and the ambient CO₂ concentration of 0.03%.

Figure 3: Graph of growth rate of *C. reinhardtii* grown in different concentrations of CO₂ for a growing period of eight days. Error bars represent standard deviation.



Urea showed a very remarkable effect on growth rate of the alga. As shown in figure 4 below there was significant difference ($df=2$, $F=13.162$, $p<0.05$) in the growth rate of the alga grown in 2.4 g/l urea, 1.8 g/l urea and 1.2 g/l urea. Grown in 2.4 g/l urea, it grew well reaching a very high stationary phase cell yield of 3.79909×10^7 after three weeks of growth. Those grown in 1.2 g/l reached a maximum cell yield of 1.09002×10^7 at the stationary phase while those grown in 1.8 g/l had a cell yield of 1.7999×10^7 at the stationary phase.

Figure 4: Cell counts for the alga grown in different concentrations of urea for the entire growing period of 23 days. Error bars represent standard deviation.

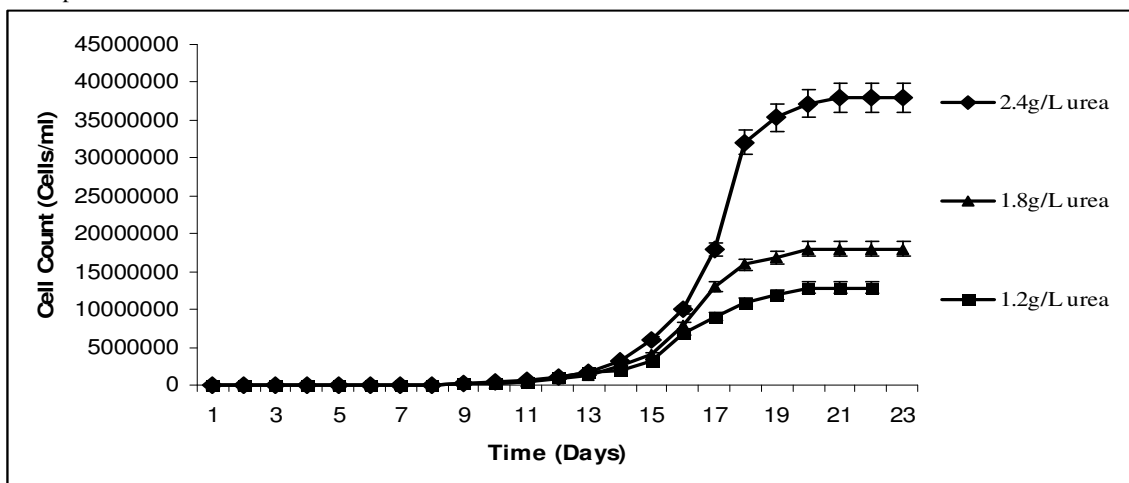


Figure 5: Comparison of the dry weights of *C. reinhardtii* grown under different concentrations of urea. Error bars represent standard deviation.

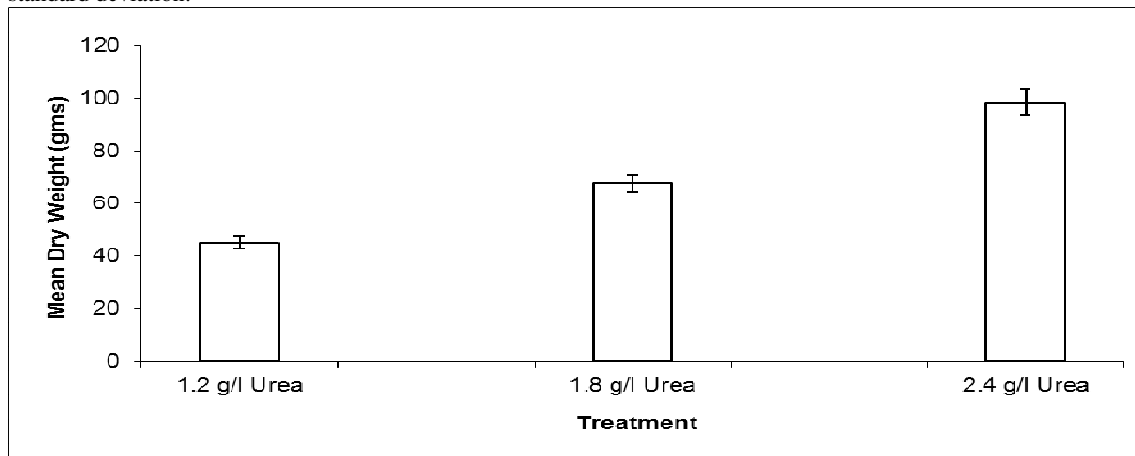
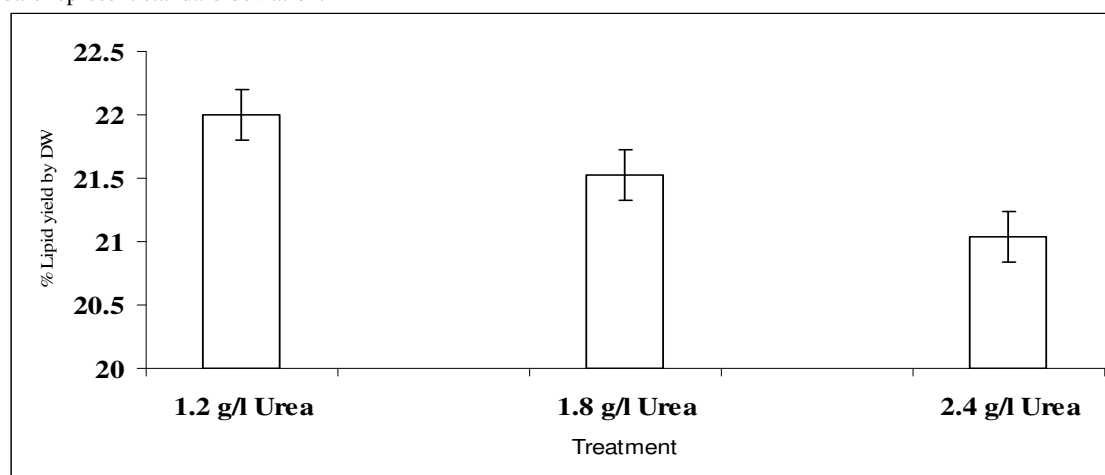


Figure 6: Lipid yield of *C. reinhardtii* grown in 1.2g/l, 1.8g/l and 2.4g/l urea expressed as percent of the dry weight. Error bars represent standard deviation.



5. Discussion

Falkowski *et al.*, (1985) observed that light influences photosynthesis and is an important factor in the growth of algae. As such, the efficiency of microalgal culture is controlled by the intensity of light and photoperiod, the two important parameters which, at optimum levels, enable the microalgae to produce energy-rich carbon compounds which can be harnessed for bio-energy production (Casadevall *et al.*, 1985). The results obtained in this study indicated that as light availability increased, so did the algal biomass. Indeed, the day length influences photosynthesis, respiration, cellular division, and the growth rate as indicated by enhanced growth at higher times of irradiation. These results are supported by the findings of Hobson *et al.*, (1979) who found that day length factor has a positive affect on the enzymatic activities and macromolecule syntheses, therefore resulting in high biomass yield. It was generally noted that the alga exhibited a growth rate that was proportional to the duration of the effective photoperiod. It would be expected, consequently, that continuous illumination would achieve the maximum growth rate recorded. Foy & Gibson (1993); Foy *et al.*, 1976 have reported this to be

actually true. However, most works generally suggest the use of light/dark cycles instead of continuous light, which seems to be inappropriate because the necessity of a dark phase is explained by the photosynthesis being governed by two reactions, a photochemical phase that is light dependent and another, a biochemical dark phase that is light independent. Similarly, light intensity played an important role in the culture of *Chlamydomonas reinhardtii*. The results show light intensities outside $160 \mu \text{mol m}^{-2} \text{s}^{-1}$ and $240 \mu \text{mol m}^{-2} \text{s}^{-1}$ ranges cannot sustain the maximum growth rate. At higher light intensities beyond $240 \mu \text{mol m}^{-2} \text{s}^{-1}$, the growth was limited due to photo-inhibition. The results further imply that this alga requires relatively low light intensities to attain a high biomass yield, an observation which was also noted by Myers (1953) for the light-saturating intensity for *Chlorella pyrenoidosa*. It was deduced from these findings that illumination intensity is one of the major parameters that should be controlled in the cultivation of the microalga since too high levels were found to cause photosystem damage, leading to low productivity whereas too low levels will cause slow growth.

Carbon dioxide is the source of carbon for the process of photosynthesis and will increase the growth rate as algal cells need it to grow autotrophically. This was very evident in the experiment on the effect of carbon dioxide on growth rate of *C. reinhardtii*. Although the supply of carbon dioxide to a mass culture system is one of the main difficulties in growing of algae according to Benemann *et al.*, (1987), it must not reach the upper concentration that it produces growth inhibition and, on the other hand, must never fall below the minimum concentration that limits growth. The alga tolerated CO_2 concentrations up to 15% exhibiting faster growth rate than when grown in the ambient concentration of 0.03%. Though the alga inhabits the waters of the Nairobi Dam where CO_2 concentration is roughly 0.03%, it was found that higher growth rate can be achieved at elevated concentrations of CO_2 .

Urea was found to have great effect on growth rate and biomass yield for the alga. Biomass growth was inhibited in nitrogen-lacking situations and higher concentrations (2.4g/l) of urea led to faster growth of the alga, reaching an enormous stationary phase cell yield of 10^7 cells/ml which upon harvesting gave the highest amount of dry weight. This observation was, however, not commensurate with the extracted lipids because there was no significant difference in amount of lipid from all the experimental groups. This was because the alga may have shifted its metabolism away from lipid production and towards cellular growth since nitrogen was available in excess, as was explained by Iwamoto and Sugimoto, (1958) who also reported similar results with different species of algae. The faster growth and subsequent low lipid yield in 2.4 g/l for *C. reinhardtii* was therefore attributed to the switch to cellular growth at the expense of lipid production leading to low lipid yield in higher concentrations of the urea.

Conclusion

From the results of these experiments it was found that *Chlamydomonas reinhardtii* is a very promising as a feedstock for bio-fuel production. It can be isolated with ease and can withstand various extreme environmental conditions and therefore was found to be the most abundant algal species inhabiting the Nairobi Dam. At a temperature of 25°C and pH of 6.9 it grows very well at illumination intensity of up to $240 \mu \text{mol m}^{-2} \text{s}^{-1}$. It can also tolerate CO_2 concentration of up to 15%, a property which can be used in sequestration of the gas from the environment. Its ability to metabolize nitrogen shows that this alga can also be used in the removal of nitrogen from wastewater.

When subjected to stress, which in this case was nitrogen limitation, it yielded lipids equivalent to 22% dry weight. As demonstrated in this research, the use of lipids from *C. reinhardtii* for bio-diesel production is feasible and production of low-cost microalgae bio-diesel primarily requires improvements to algal growth conditions. Thus, in the context of climactic changes and soaring prices of petroleum, bio-fuels from algae such as *C. reinhardtii* are now being presented as a renewable energy alternative.

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