Journal of Energy Technologies and Policy www.iiste.org
ISSN 2224-3232 (Paper) ISSN 2225-0573 (Online)
Vol.3, No.11, 2013 – Special Issue for International Conference on Energy, Environment and Sustainable Economy (EESE 2013)

Outdoor Large scale Microalgae consortium culture for biofuel production in South Africa: Overcoming adverse environmental effects on microalgal growth

Maranda Esterhuizen-Londt Ben Zeelie

InnoVenton/DCTS; Nelson Mandela Metropolitan University; Summerstrand Campus (North); University Way; Summerstrand; Port Elizabeth; 6001; SOUTH AFRICA

*Email address of corresponding author: maranda.esterhuizen2@nmmu.ac.za

Abstract

In nature, microalgal blooms occur regularly and in contrast with laboratory cultivation procedures, these blooms are not axenic and this seems to add to the longevity and intensity of the bloom. For this reason a consortium of microalgae and bacteria is used at InnoVenton for large scale biomass cultivation. The biomass produced, has successfully been used to produce biocrude. Laboratory cultivation procedures also require a large energy input in terms of artificial lighting, heating and aeration making it a costly endeavour. However, this stringent control of the culture to avoid contamination and ensure optimal growth conditions is essential when cultivating the microalgae for medical and pharmaceutical applications. When culturing for a chemical application such as biofuel production, this is not the case, therefore allowing for the economical outdoor consortium cultivation approach employed at InnoVenton. In this study we investigated whether morning heating of the media will overcome low consortium growth rates experienced in winter and whether the use of glucose, ethanol and acetate will overcome biomass loss exhibited at night. The results showed that heating increased the growth rate relative to those measured in summer and that heating all day in winter did not induce better growth rates in comparison to only heating for an hour at sunrise. We show that all three carbon sources are efficient at overcoming biomass loss at night with glucose being the most effective. In conclusion, employing these two techniques, the same growth rate theoretically can be achieved year round with large scale outdoor cultivation.

Keywords: consortium, microalgae, biocrude

1. Introduction

Large scale microalgal cultivation is constantly critiqued due to its high costs and is therefore not a front contender for use in biofuel production (Christi, 2007; Wijffels and Barbosa, 2010; Wu et al., 2012; Greenwell et al., 2012). Current cultivation methods are based on laboratory techniques essential for ensuring contaminant free biomass for medical and pharmaceutical applications (Christi, 2007). However, for a chemical process application such as biofuel production, this is not necessary and the cultivation costs can therefore be reduced with outdoor cultivation employing ambient light and heating. This can be very efficient when cultivation takes place in a country such as South Africa with such temperate seasons. In nature, blooms occur naturally and are in no way axenic. The symbiotic relationship of the algae with the bacteria seems to prolong bloom events and increase the bloom density (Fukami et al., 1997). InnoVenton therefore employs such a large scale cultivation system consisting of a consortium of microalgae and bacteria with demonstrated ability to produce biocrude from the biomass (Esterhuizen-Londt and Zeelie, in press). However, even though the seasons experienced in South Africa are mild, it still influence the growth rates of the consortium and together with night biomass loss, this allows opportunity for enhancement of the annual biomass turnover.

1.1 Heating

Daily circadian rhythms coordinate and monitor physiological events in microalgae so that metabolism, physiology and behavioural patterns occur at optimal phases of the diurnal cycle. Circadian rhythms are temperature compensated (Suzuki and Johnson, 2001) and thus the environmental temperature will have a direct impact on microalgae growth. In general, temperature is the major regulating factor in cell regulation, morphological and physiological responses of microalgae (Zeng et al., 2011). Cell growth rate or division is directly proportional to temperature. This stems from optimal temperatures required for enzymes involved in metabolism and physiological responses. Optimal temperatures for microalgae growth range from 25 to 35^{o}C with a maximum cell growth rate generally around 30^{o}C with the minimal temperature for supporting microalgal growth recorded at 16^{o}C (Converti et al., 2009). However, optimal temperature is also affected by other factors such as light intensity and humidity. Therefore, microalgae growth rate in an environmental setting will be

affected by daily and season temperatures and thus the length of the day with temperatures above 16°C. It has been shown that the eukaryotic green microalgae *Dunaliella* sp. displays biomass accumulation proportional to the length of the photoperiod (Foy et al., 1976).

It is important to sustain microalgae cultures with a high photosynthetic capacity since this will result in a higher biomass yield. Respiration and photorespiration are wasteful processes and will lead to a loss in biomass yield and should thus be avoided or essentially kept to a minimum. Temperature has a strong effect on these processes when CO_2 and/or light is limiting. High temperatures will lead to increased respiration (Ogbanna and Tanaka, 1996); thus decreasing the efficiency of photosynthesis as more energy is expended then produced (Pulz, 2001).

1.3 Night biomass loss

Microalgae photosynthesize to produce carbohydrates and adenosine triphosphate (ATP) needed for cell growth and reproduction. When the light intensity is too low to support photosynthesis, cells do not grow but respire to remain viable and thus leading to reduced biomass. It has been reported that as much as 35% of the biomass produced during the day can be lost at night (Grobelaar and Soeder, 1985; Torzilla et al., 1991; Ogbanna and Tanaka, 1996). Ogbanna and Tanaka (1996) reported that during the night the carbohydrate concentration decreased, however, the protein content increased thus intracellular carbohydrate stores are utilized.

Nightly biomass loss depends on the daily light level, light period temperature and the temperature at night. Ogbanna and Tanaka (1996) reported that the biomass lost at night decreased with higher daytime temperatures but increased with high light intensity. In their attempts to overcome biomass loss Ogbanna and Tanaka (1996) reported that it could not be completely circumvented but only reduced by decreasing the temperature and avoiding mixing at night. Ogbanna and Tanaka (1996) successfully overcame biomass loss at laboratory scale by the addition of glucose, acetate or ethanol to their cultures thereby achieving continuous growth. They reported that acetate and ethanol was preferred in order to avoid the risk of contamination.

1.4 Research Aim

The aim of this study was to determine whether supplying exogenous heat at daybreak to raise the temperature above 16°C is effective to increase the effective photogenic period during winter months. We also investigate the effect of the three organic carbon sources glucose, sodium acetate and ethanol on the microalgae consortium grown outdoors in a greenhouse at large scale in order to avoid biomass loss at night. Seeing as harvesting does not take place on a daily basis at InnoVenton, this will lead to increased biomass yield.

2. Method

2.1 Cultivation details

The microalgae consortium dominated by eukaryotic *Scenedesmus* sp., *Chlamydomonas* sp. and *Chlorella* sp., and cyanobacteria including *Limnothirix* sp. were obtained from various sources, combined with naturally occurring symbiotic bacterial were cultured non-sterilely over a year period for the communities to establish and stabilize. The consortium was cultured in vertical enclosed photobioreactors (PBR, Figure 1) with airlift sparging supplemented with 5% CO_2 in the InnoVenton greenhouse.

Figure 1: Image showing the InnoVenton greenhouse with the PBR setup and placement.

The PBRs were solely a function of the environmental temperature and season unless stated otherwise. The mixed consortium of microalgae, cyanobacteria and heterotrophic bacteria were fed with a feed mixture based on the carbon, hydrogen, nitrogen, and sulphur (CHNS) analysis of the consortium (Esterhuizen-Londt and Zeelie, in press).

2.2 Heating

The standard consortium was used to determine whether increasing the temperature of the culture media at the start of the photoactive period (early morning) affected the biomass accumulation on a daily basis. The heterogeneous culture was inoculated into tap water and fed once daily. The pH of the culture was controlled to start at 7.5.

The treatment consisted of a PBR (in triplicate) partially suspended in heated water (30^{o}C) and the control consisted of a PBR partially suspended in water at ambient temperature. In one set of experiments, the treatment PBRs were heated at 30^{o}C for the entire photoperiod and for the other set, the treatment PBRs were only heated at the start of the photoperiod for one hour (to ensure that the culture medium's temperature was raised to above 16^{o}C). The experiment was replicated over three days to account for environmental variation. The biomass growth was measured as increase in turbidity with a TB 200 turbidity meter (Orbeco) and the pH and temperature was monitored with a Hanna pH and temperature meter at the start, mid-point and at the end of the photoperiod. It should be noted that the relationship between turbidity and biomass as dryweight is not linear.

Statistical analysis was conducted using Statistica 2010^{TM}. The analysis of variance (ANOVA) test was used to determine significant differences between each treatment set and its corresponding control.

2.3 Night biomass loss

For the mixotrophic growth experiment, six PBRs were used with random placement in the InnoVenton Greenhouse. The microalgae consortium was operated in fed-batch mode with an approximate starting density of 0.5 g DW L^{-1}.Each treatment (glucose, sodium acetate and ethanol) was conducted in a separate PBR with its own corresponding control. Each PBR was sparged with air supplemented with 5% CO_2 during the entire photoperiod. The cultures were fed with the normal feed stock daily; however, the pH was altered only on day 1 to approx. 7.5 using acetic acid to avoid any unnecessary carbon addition. The microalgae were left to grow during daylight hours and before sunset the glucose, acetate and ethanol was added according to the uptake rate and cell density per respective PBR (Ogbanna and Tanaka, 1996). The microalgae growth was measured as a function of turbidity (TB 200 turbidity meter, Orbeco) and pH and temperature was measured (Hanna meter) over the period of 84 hours (3 dark periods included). This was repeated three times to be able to account for the variation in environmental conditions.

Statistical analysis was conducted in Statisica 2010^{TM}. Regression analysis with indicator variables was used to differentiate between the indicators and the control treatments.

3. Results and Discussion

3.1 Heating

Environmental culture compared to laboratory culture in a closed controlled system, will usually have more losses in biomass due to sedimentation, grazing, parasitism and general loss. It is nearly impossible to determine these losses and thus the growth rate or biomass yield should be seen as a minimum value. The biomass accumulation per day is thus used as an indication of growth.

Figure 2A and B shows there is a significant difference between the growth of the heated PBRs versus the controls for each day 1 ($p<0.05$). For all heated treatment there was an overall increased growth compared to the unheated control. From figure 2, the variation in environmental conditions is evident by the difference in biomass accumulation seen for each day. However, irrespective of the day, the biomass yield is larger for the heated reactors compared to the corresponding control. Thus, heating for the entire light period or just an hour at the beginning of the light period essentially results in increased growth. It can be concluded that in general, heating to above the minimal growth temperature of microalgae, increases the available photoperiod allowing for longer photosynthesis, thereby increasing the biomass accumulation per day during winter months. Due to the excess cost associated with prolonged heating it is suggested that 1 hour heating is implemented during the winter months. As heating can be attained as waste heat from factories in the form of flue gas, this may be implemented at minimal or no cost. The two sets of experiments were not conducted on the same day and therefore cannot be directly compared due to daily variations in environmental conditions.

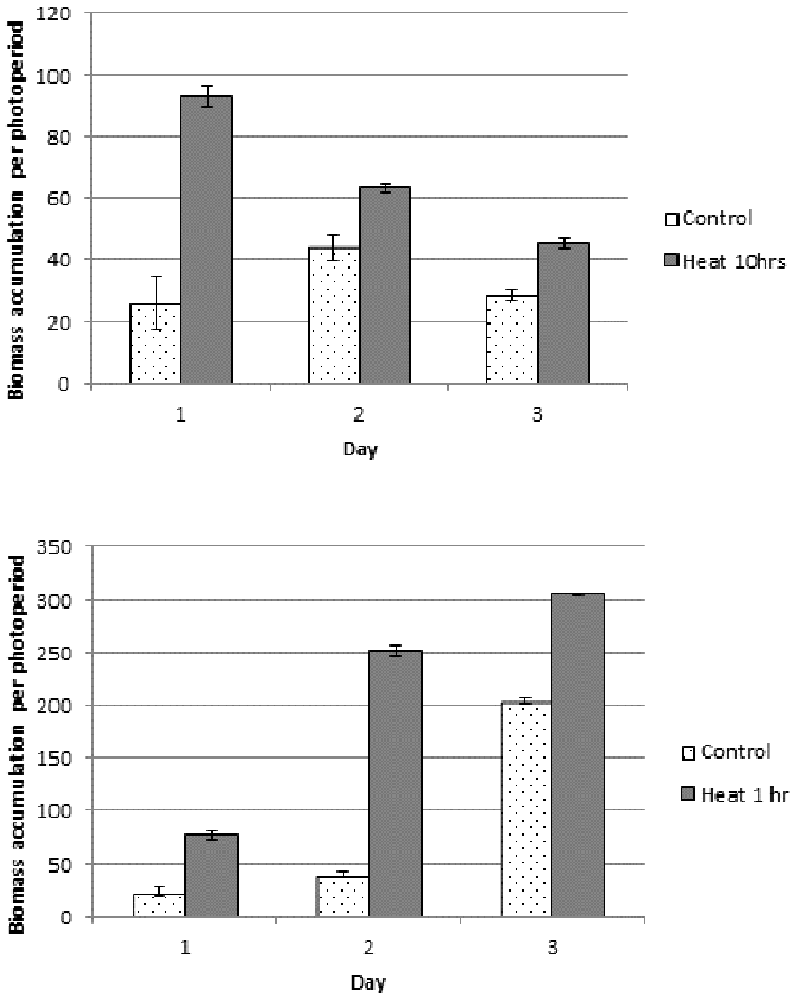

Figure 2: Biomass gain on the three replicate days A) PBRs heated at 30°C for the entire photoperiod. B) PBRs heated at 30°C for an hour in the morning to raise the media temperature above 16°C. For all data error bars denote standard error (*N*=3).

3.2 Biomass Loss

For all controls, a typical oscillating growth pattern is seen (Figure 3). This trend is seen because the microalgae photosynthesis during the day and respire during the night.

With the addition of glucose, the same pattern is not seen as the growth seems to smooth out; this is due to heterotrophic metabolism at night. For both acetate and ethanol the smoothing out is also seen initially however it is not sustained over the 3 day period and respiration is seen from night 2 onwards. This indicates either that these are not preferred carbon sources which contradict literature or that the concentrations of these carbon sources were not sufficient to support heterotrophic growth during the dark period.

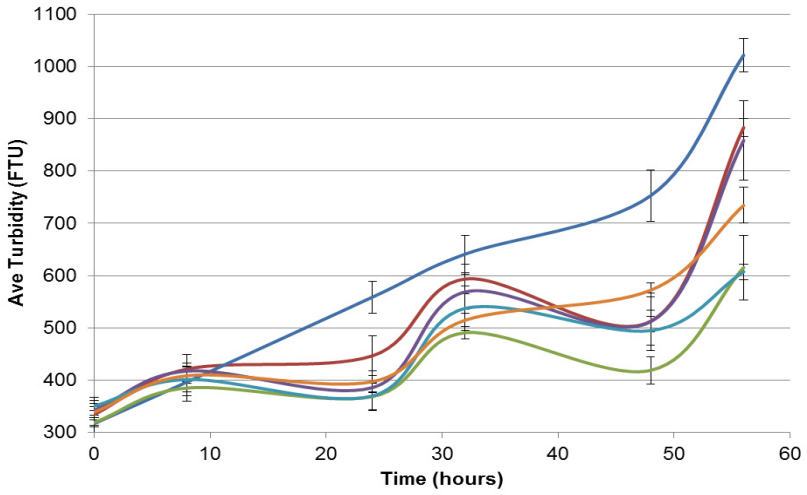

Figure 3: The average growth of the microalgae over time for the various treatments and their corresponding controls. G , glucose treatment; CG , control corresponding to the position of the glucose treated PBR; E , ethanol treatment; CE, control for ethanol treatment, A, acetate; and CA, control for the acetate treatment. Error bars denote standard error (*N*=9)

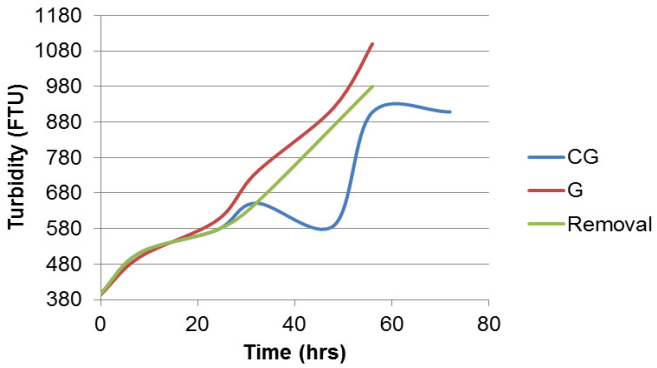

Figure 4: The average growth of the microalgae over time with glucose addition and its control. The green line (Removal) represents the controls growth minus the biomass loss at night (calculated) to compare it to growth with glucose addition.

Figure 4 compares the growth rate of the glucose supplemented microalgae to the control (minus the dark phase biomass loss) to investigate whether there is actual growth during this phase or if the biomass is only being sustained and respiration avoided. It can be deduced that glucose only sustains the biomass concentration during the dark phase and does not promote growth.

In all the tested treatments, the addition of a carbon source during the dark phase resulted in an overall increase in the growth rate and thus increased biomass yield (Figure 5). The average rate of increase for glucose treated microalgae is 3.57 turbidity units per hour higher compared to the control with is significantly higher (p=0.0001). There is some evidence that the growth rate increase (turbidity is 1.24 units/h higher) when microalgae is treated with acetated compared to its corresponding control (p=0.053). The average rate of increase for the microalgal consortium supplied with ethanol during the dark period is 2.35 units/hour higher than that of the control (p=0.006). No excessive bacterial growth was observed, however this was not specifically monitored. It may be that only enough glucose was added for microalgal uptake as the uptake rate per microalgal mass was calculated.

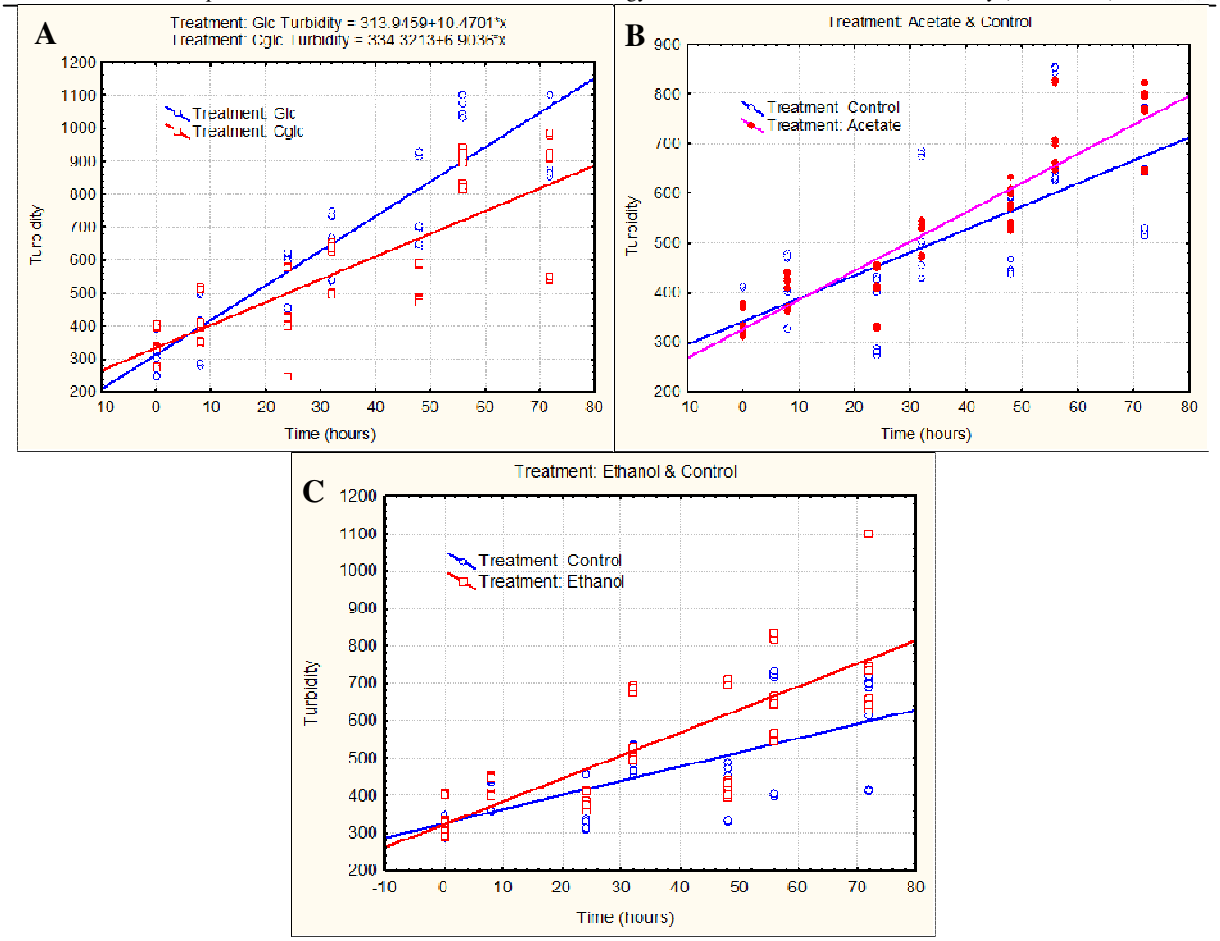

Figure 5: Growth of the microalgae per treatment over time compared to its corresponding control. A. Treatment with glucose; B treatment with Acetate and C. treatment with Ethanol (*N*=9).

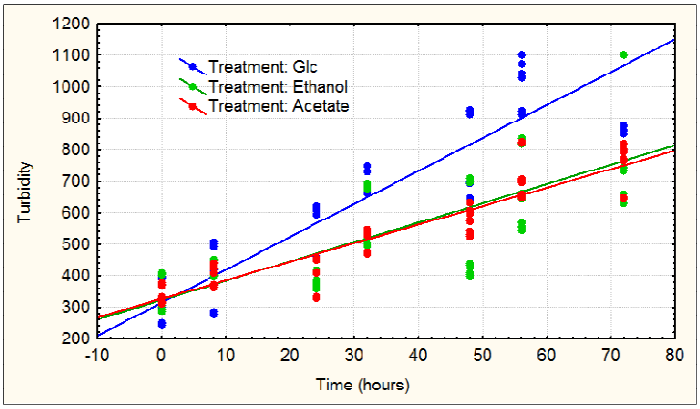

Figure 6: Comparison of the growth of the microalgae with the 3 treatments used to overcome night biomass loss (*N*=9).

Overall, it can be deduced from Figure 6 that when comparing the three carbon sources for heterotrophic growth to overcome night biomass loss, glucose results in a significantly higher growth rate.

Even at large scale with consortium culture, heterotrophic microalgal growth on extracellular organic carbon sources results in the successful reduction of night biomass loss due to respiration. It can be concluded that glucose is the better of the three carbon sources tested. No increase in bacterial growth was observed but this should be tested in future.

In conclusion, with the implementation of the two techniques, theoretically constant year round turnover can be achieved when growing a microalgal consortium outdoors at large scale with reduced cost compared to axenic

cultivation. However, addition of a carbon source may become impractical at commercial scale due to the masses required.

Acknowledgements

The authors would like to thank Mr C. Bosma for assistance with statistical analysis of the data as well as the Technology Innovation Agency (TIA), the Department of Science and Technology (DST), c*change, THRIP and ESKOM for funding the research.

References

Christi, Y. 2007. Biodiesel from microalgae. Biotechnology Advances 25 (3), 294–306.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and M.D. Borghi. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochlorpsis oculata* and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing 48, 1146-1151.

Esterhuizen-Londt, M. and B. Zeelie. In Press. Large scale cultivation of a mixed microbial consortium for biofuel production In: Industrial Biotechnology in Non Aligned and Developing Countries: Current Status and Future Prospects. NAM S&T, India.

Fukami, K., Nishijima, T. and Y. Ishida. 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Developments in Hydrobiology 124, 185-191.

Grobelaar, J.U. and C.J. Soeder. 1985. Respiration losses in green alga cultivated raceway ponds. J. Plankton Res., 7, 497-506.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J. Lovitt, R.W. and K.J. Flynn. 2012. Placing microalgae on the biofuels priority list. J.R. Soc Interface 7, 703-726.

Ogbanna, J.C. and H.Tanaka. 1996. Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of Chlorella pyrenoidosa. J Ferment Bioeng 82 (6), 558-564.

Perez-Garcia, O. Escalante, F.M.E., deBashan L.E. and Y. Bashan. 2011. Review: Heterotrophic cutures of microalgae: Metabolism and potential products. Water Research 45, 11-36.

Pulz, O. 2001. Photobioreactors: production systems for phototrophic microorganisms. Appl. Microbiol. Biotechnol. 57, 287-293.

Suzuki, L. and C.H. Johnson. 2001. Minireview: Algae know the time of day: Circadian and Photoperiodic programs. J. Phycol. 37, 933-942.

Torzilla, G., Sacchi, A., Materassi, R., and A. Richmond. 1996. Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. J. Appl. Phycol., 3, 103-109.

Wijffels, R.H. and M.J. Barbosa. 2010. An Outlook on Microalgal Biofuels. Science 329 (5993), 796 - 799.

Wu, L.F., Chen, P.C., Huang, A.P. and C.M. Lee. 2012. The feasibility of biodiesel production by microalgae using industrial wastewater. Bioresource Technology 113:14-8.

Zeng, X, Danquah, M.L., Chen, X.D. and Y. Lu. 2011. Microalgae bioengineering: From CO_2 fixation to biofuel production. Renewable and sustainable energy reviews 15, 3253-3260.