International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-2539, Volume 3, Issue 4; October-December, 2016, pp. 252-257

http://www.krishisanskriti.org/Publication.html

Enhancing Lipid Yield for Biodiesel Production using Microalgae: *Chlorella sorokiniana*

Ashok Meena, Sukhendra Singh* and Subir Kundu

School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University) Varanasi-221005
*E-mail: *skundu.bce@itbhu.ac.in*

Abstract—*Economic growth and increased standards of living given rise to increased demand for energetic sources. In the last decades, fossil fuels have been widely used as a source of energy. Subsequently, now we have to think about the low availability of this non-renewable energy resource and about the environmental effects associated with the use of fossil fuels. The depletion of fossil fuel reserves has inspired the search for sustainable sources of energy that are carbon-neutral or renewable. In this context, Microalgae are a promising, energetic resource. Microalgae are best known for their high lipid contents and high growth rates, being a promising oil source for biodiesel production. The lipid content for microalgae varies from 20% to 50% of dry weight mass. Microalgae are photosynthetic microorganisms that use CO2 as a carbon source, with high specific growth rates. Furthermore, Cultivation conditions (nutrient medium, temperature, pH, nutrient starvation, salinity, aerating) are the major factors that influence photosynthesis activity and behavior of the microalgae growth rate. In this study, we investigated the effect of cultivation conditions on microalgae growth and lipid productivity of Chlorella sorokiniana. For this first, we checked the effect of pH and different media. Chlorella sorokiniana is simultaneously grown in three different media at three different pH. Bold's Basal Medium, TAP Medium, and BG-11 are separately used at three different pH 6, pH 7 and pH 8 for growth. The growth of Chlorella sorokiniana was measured at wavelength of 688 nm. After 14 days cell biomass was harvested and lipid concentration was measured using gas chromatography. It is noticed that TAP media gives highest lipid content at pH-8.0. Afterward, the effect of other factors such as salinity, nitrogen starvation, and phosphorus starvation was also observed for lipid productivity of Chlorella sorokiniana.*

Keywords*: Microalgae, Biodiesel, Gas chromatography*

1. INTRODUCTION

Sustainable production of renewable energy is being debated worldwide since it is increasingly understood that first generation biofuels, mainly produced from food crops and commonly oil seeds, contest for arable land, freshwater or bio diverse natural lands and are restricted in their ability to accomplish goals for biofuel production. These concerns have enlarged the interest in developing and such as lignocelluloses, the second generation biofuel and microalgae, the third generation biofuels, which possibly offer great prospects in the longer term and do not need to contest for arable land and valuable freshwater [1,2]. Due to constant and rising use of fossil fuels, the extent of greenhouse gas CO2 has increased. As an outcome global warming and climate change are intimidating ecological stability, food security and social welfare [3, 4]. The transport and energy section are the two major sources, accountable for the generation of 20% and 60% of greenhouse gases releases, respectively, and it is imaginable that with the growth of emerging countries the global consumption of energy will rise significantly and this will lead to extra environmental damage [5]. Photosynthesis is the single process that can transform carbon dioxide into organic compounds with high energy content, and thus can give a source for viable transport fuel production. There is a serious need to improve technologies which are able to produce an extra five to six billion tons of organic carbon apart from the present harvest from agricultural crops [3]. Rather than other biofuel crop, cultivation of microalgae may be 10–20 times more productive on a per hectare on large-scale, which are able to use a wide-ranging variety of water sources, and have a solid potential to make biofuels without the struggle for food production [2]. Algae can be grown either in large-scale microalgae cultivation systems in open ponds or in photo bioreactors [1] or as macrophytes through marine aquaculture [6]. Microalgae are now considered the most favorable types of algae for biofuel production, due to their high lipid contents.

Recent research in the production of microalgae has been intensively studied [7], and future outlooks have been given by Stephens et al. [5]. Microalgae can also be cultivated as an additional concept with wastewater treatment to increase the energetic and financial input for the production process [8]. Triacylglycerides (TAGs) generally used as energy storage in microalgae. After extraction that can be easily converted into biodiesel through transesterification process [3, 9]. These neutral lipids have a common structure of three esters where generally three long-chain fatty acids (FAs) are coupled to a glycerol molecule. Transesterification converts glycerol into small alcohols like methanol. In recent times, the increase in petroleum prices and the need to decrease greenhouse gas

emission has seen a new interest in large-scale production of biodiesel [10]. In the last few decades the idea of lipid induction in microalgae has been intensively studied to increase triacylglycerides production in microalgae, but now different lipid induction methods have not been compared to each other. Here we give a review of different lipid induction methods in microalgae and their need to be used for biodiesel production [11].

2. MATERIALS AND METHODS

2.1 Growth analysis of microalgae

The *chlorella sorokiniana* microalgae sample were obtained from Bioreactor design and Cell Processing laboratory of School of Biochemical Engg., IITBHU (Varanasi).

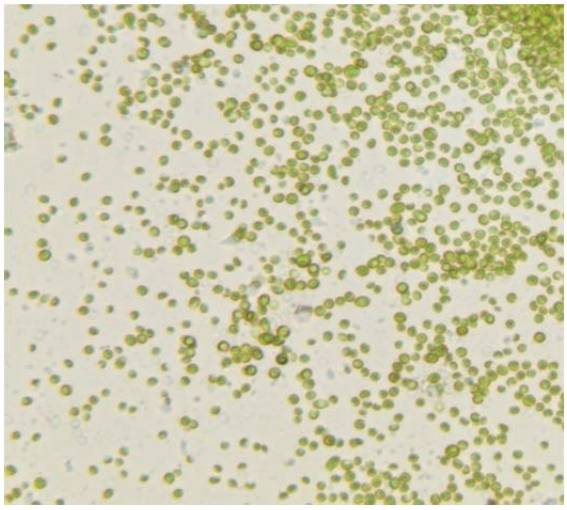


Fig. 1: *Chlorella sorokiniana* microalgae.

The three culture media used were Bolds Basal Medium (BBM), TAP medium and BG-11 medium. Each media were made in institute laboratory using composition data. Each media was used at three different pH levels (6, 7 and 8). For autotrophic cultivation, the microalga was cultured at 28°C in an orbital shaker at 90 rpm under continuous illumination. Nine 100 ml flasks were simultaneously used for growth using different media at pH 6, 7 and 8. Each 100 ml media were inoculated with 5 ml inoculum and incubated in orbital shaker having light intensity of 4000 lux and light: dark period 12:12 (h: h).

The growth analysis of microalgae culture was daily measured starting from first day using UV spectrophotometer. The absorbance was measured at 688 nm and pH were also measured at regular interval.

2.2 Lipid content analysis of microalgae

Lipid analysis is done using gravimetric method. In this, lipids are extracted from a sample, the extraction solvent is evaporated and the retained material is measured as the lipid content [12, 13].

For this, first microalga cultures were harvested and then dewatered by centrifugation. The supernatant was discarded and the microalgae pellet was taken out and lyophilized. The dried microalgae was grind into fined powder. The powder was stored at 4℃ before they used for lipid extraction. Then Soxhlet apparatus is used for further extraction.

For this Chloroform-Methanol soxhlet method was used. 210 ml of chloroform- methanol was taken in the proportion of 2:1, v/v and refluxed at a siphon rate of 6-8 times over the thimble between 12 and 20 h using a standard soxhlet apparatus. The extracted material was then transferred and made up to 250 ml with chloroform-methanol. Then transferred to aeparation funnel and a 0.7-0.75% NaCl aqueous solution was mixed with aliquot at a final ratio of 8:4:3 chloroform/methanol/NaCl aqueous to remove the nonlipid materialfrom the solvent, similar to the washing procedure described in Folch et al. The resulting two phase mixture was allowed to settle down for 12 h. After settling, the lower or organic phase containing the lipids was drained into a pre weighed flask and the solvent was taken out using vacuum rotary evaporation at 30− 35 °C. For drying the flasks were then placed in a 40 °C vacuum oven. Then lipid content was calculated using gravimetric method [14, 15].

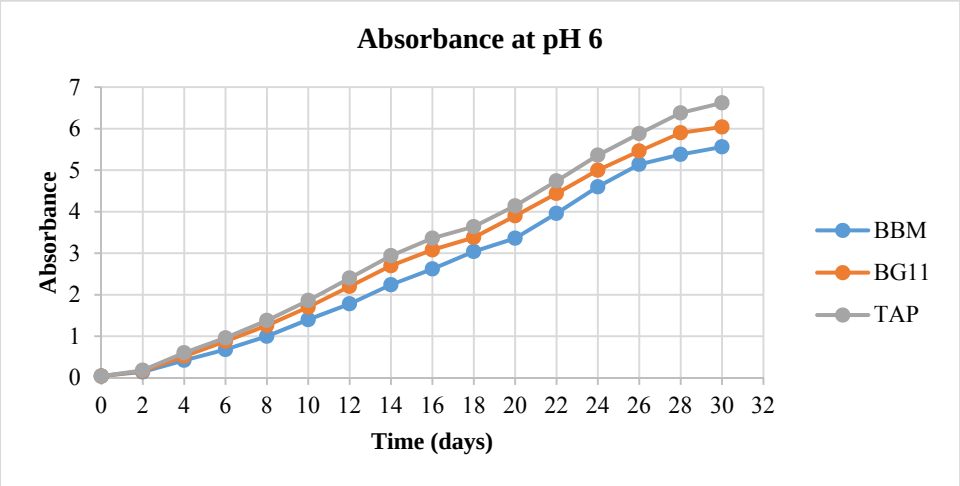
2.3 Transesterification reactions

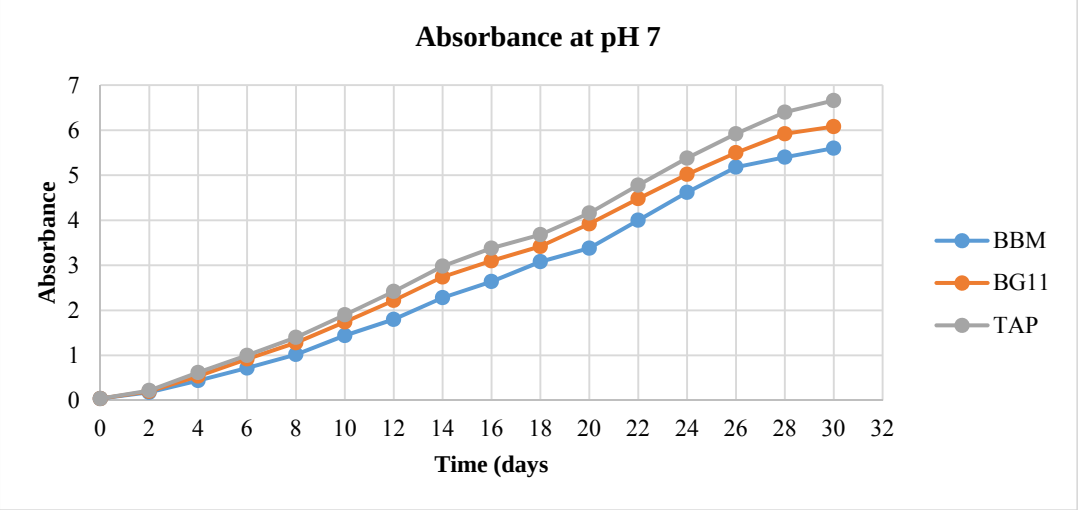
Transesterification is a multistep reaction, which includes three reversible steps in series, where triglycerides are converted to di-glycerides, then di-glycerides are converted to mono glycerides, and mono glycerides are then converted to esters (biodiesel) and glycerol (by-product).

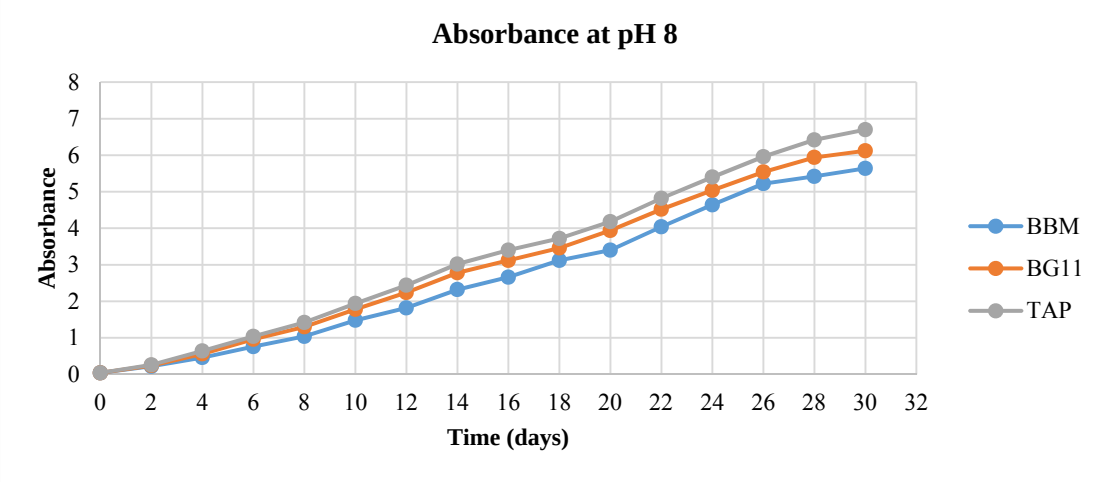
Transesterification was done on the lipid which was previously extracted from 3 grams of dry microalgae mass with C/M using Soxhlet apparatus. A vessel equipped with a condenser was used and the extracted oil was charged with the alkoxide solution of KOH alkali catalyst and methanol. Methanol to algal biomass v/w ratios was (8:1, 12:1 and 16:1). The mixture was then heated to a temperature of 60^{o}C. Then reaction was allowed to take place. The vessel was then cooled to room temperature to stop the reaction. Phase separation was done following the technique of Folch et al. (1957) [14]. For this chloroform and water were added into the mixture and the ratio of mixture: chloroform: water of 10:10:9. After shaken strongly, the mixture was centrifuged at 2000 rpm for 10 min, separates it into two phases. Fatty acid methyl esters, free fatty acids, lipids and other non-polar compounds well dissolved in the chloroform bottom phase while methanol and other polar impurities that were dissolved in water formed the upper phase, which was collected for further analysis.

3. RESULTS

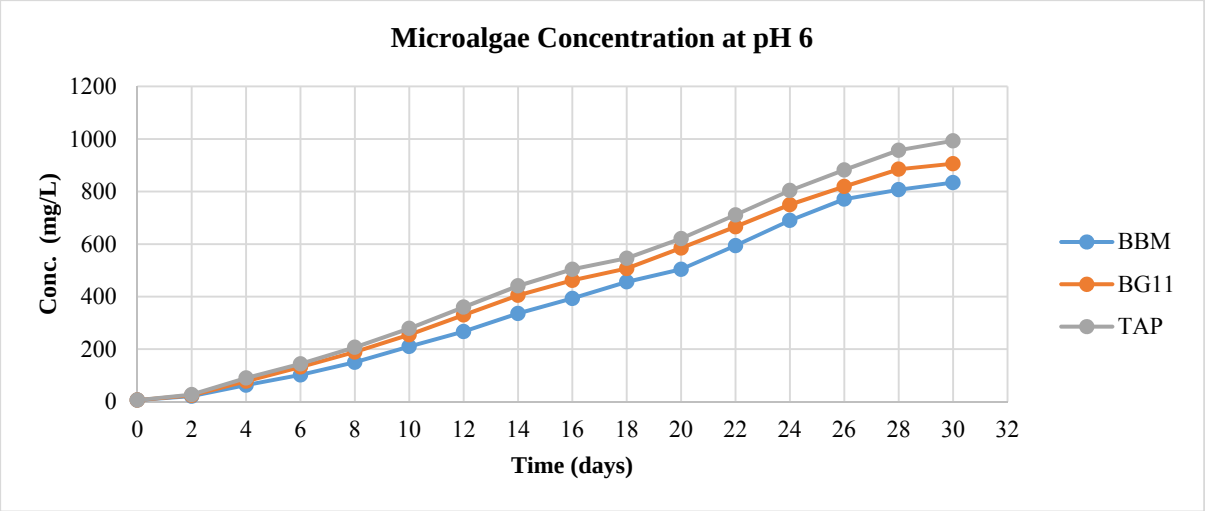
The growth curves were plotted at pH 6, 7 and 8 for three media BBM, BG11 and TAP using absorbance which was taken after 20 times dilution.

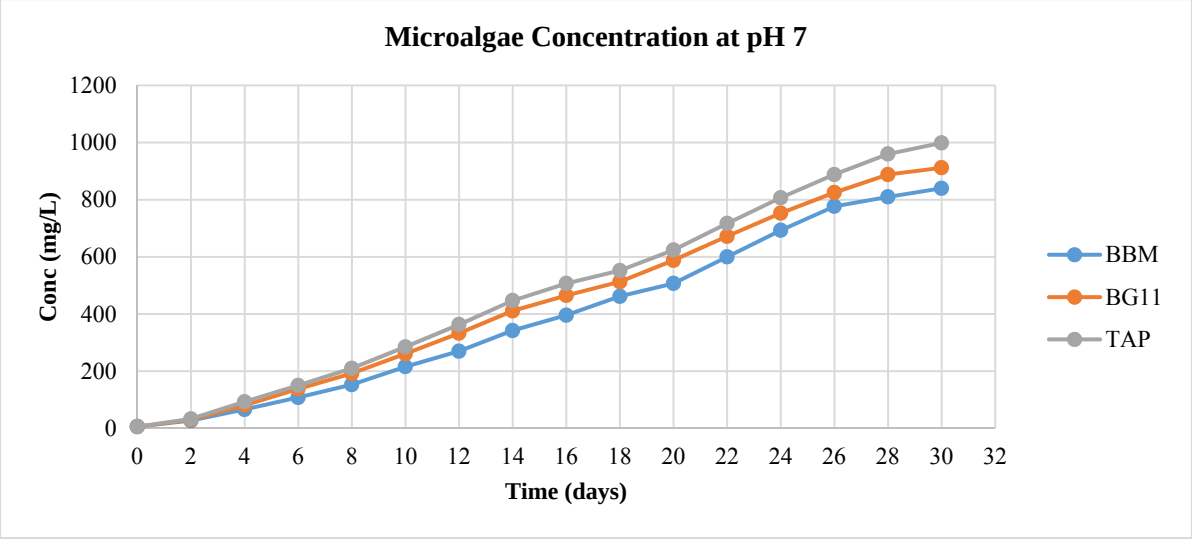


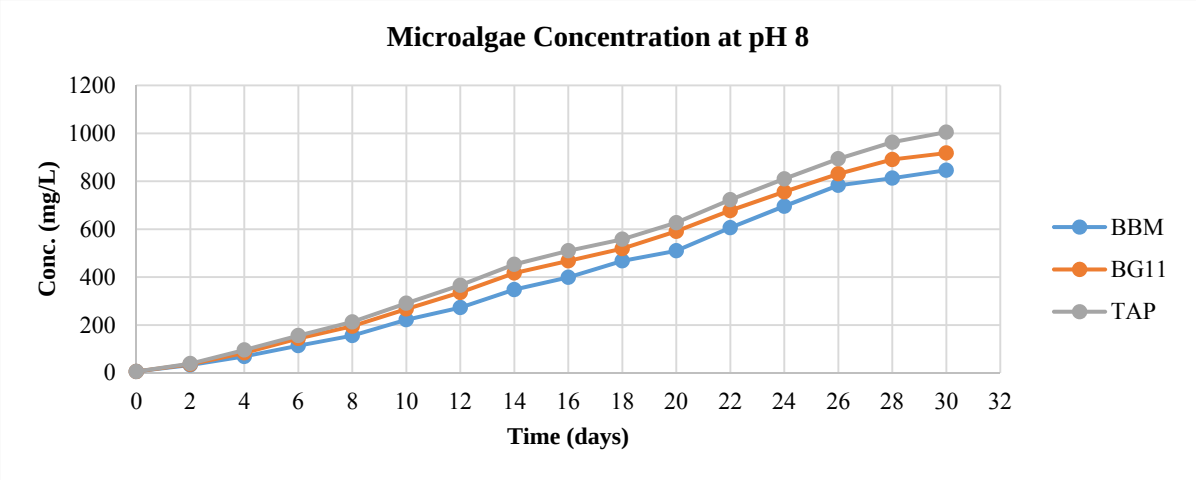




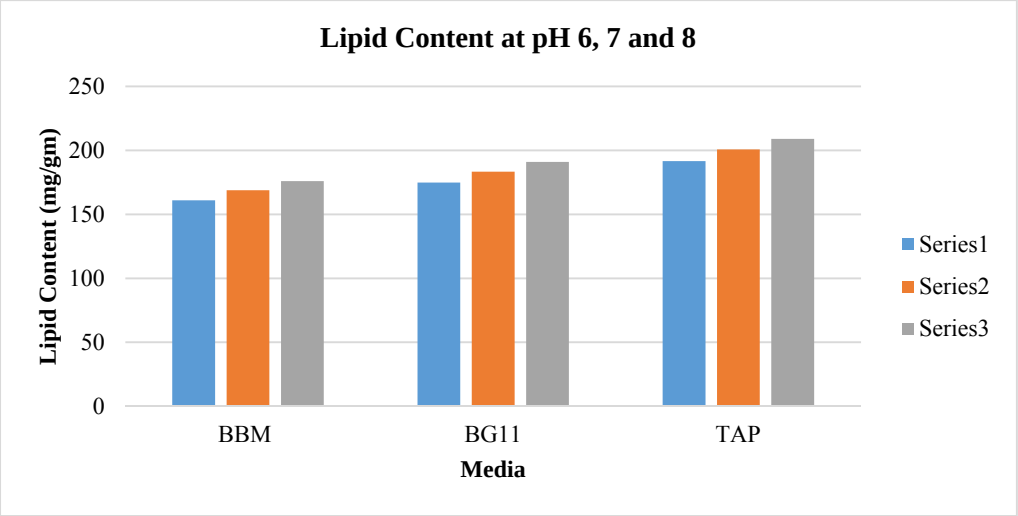
Further curves were plotted showing microalgae concentration (mg/L) at pH 6, 7 and 8 for three media BBM, BG11 and TAP.







Lipid content was calculated which was 20.1 ±0.7 % of dry weight. The lipid contents for all three media at different pH conditions was plotted.



Afterwards, biomass productivity and lipid prductivity was calculated using

$P_{Biomass}$ ($gL^{-1}day^{-1}$) = $(X_2 - X_1)(t_2 - t_1)^{-1}$

where X_1 and X_2 were the biomass dry weight concentrations (g L^{-1}) and t_1 is startpoint of cultivation and t_2 is endpoint of cultivation.

P_{lipids} ($mgL^{-1}day^{-1}$) = $P_{Biomass}$ * C_f

where P_{lipids} is the lipid productivity; $P_{Biomass}$ is biomass productivity; and C_f is the final lipid content and were given as percent dry weight [16, 17].

Table 1: Biomass and Lipid Productivity.

Nutrient Media	pH of medium	$P_{Biomass}$ ($g.L^{-1}.d^{-1}$)	P_{Lipid} ($mg.L^{-1}.d^{-1}$)
Bolds Basal Medium	6.0	27.2	4.4
	7.0	27.8	4.7
	8.0	28.0	5.0
BG-11 Medium	6.0	30.0	5.2
	7.0	30.2	5.5
	8.0	30.4	5.8
TAP Medium	6.0	32.9	6.3
	7.0	33.1	6.6
	8.0	33.9	7.0

4. CONCLUSION

The growth curves data shows that *chlorella sorokiniana* gives highest lipid yield using TAP media at higher pH values (pH 8). Also lipid productivity and biomass productivity was high for higher pH values of nutrient media. Therefore, this can be concluded that microalgae *Chlorella sorokiniana* can be used as a renewable source for biodiesel production.

REFERENCES

[1] Schenk, P.M.; Thomas-Hall, S.R.; Stephens, E.; Marx, U.; Mussgnug, J.; Posten, C.; Kruse, O.; Hankamer, B. Second generation biofuels: High-efficiency microalgae for biodiesel production. BioEnergy Res. 2008, 1, 20–43.

[2] Chisti, Y. Biodiesel from microalgae. Biotechnol. Adv. 2007, 25, 294–306.

[3] Chisti, Y. Biodiesel from microalgae beats bioethanol. Trends Biotech. 2008, 26, 126–131.

[4] Christenson, L.; Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 2011, 29, 686–702.

[5] Stephens, E.; Ross, I.L.; Mussgnug, J.H.; Wagner, L.D.; Borowitzka, M.A.; Posten, C.; Kruse, O.; Hankamer, B. Future prospects of microalgal biofuel production systems. Trends Plant Sci. 2010, 15, 554–564.

[6] Bruton, T.; Lyons, H.; Lerat, Y.; Stanley, M.; Rasmussen, M.B. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland; Technical Report; Sustainable Energy Ireland: Dublin, Ireland, 2009.

[7] Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 2010, 14, 557–577.

[8] McGinn, P.; Dickinson, K.; Bhatti, S.; Frigon, J.-C.; Guiot, S.; O'Leary, S. Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: Opportunities and limitations. Photosynth. Res. 2011, 109, 231–247.

[9] Fukuda, H.; Kondo, A.; Noda, H. Biodiesel fuel production by transesterification of oils. J. Biosci. Bioeng. 2001, 92, 405–416.

[10] Chen, Y.F. Production of Biodiesel from Algal Biomass: Current Perspectives and Future; Academic Press: Waltham, MA, USA, 2011; p. 399.

[11] Kalpesh K. Sharma, Holger Schuhmann and Peer M. Schenk High Lipid Induction in Microalgae for Biodiesel Production; energies ISSN 1996-1073; p. 5

[12] Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917

[13] Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues.J Biol Chem 226(1):497–509

[14] Lieve M. L. Laurens , Thomas A. Dempster , Howland D. T. Jones , Edward J. Wolfrum , Stefanie Van Wychen , Jordan S. P. McAllister , MichelleRencenberger, Kylea J. Parchert , and Lindsey M. Gloe Algal Biomass Constituent Analysis: Method Uncertainties and Investigation of the Underlying Measuring Chemistries; p. 2

[15] Folch, J.; Lees, M.; Sloane-Stanley, G. H. J. Biol. Chem. 1957, 226, 497−509.

[16] Kumar K1, Dasgupta CN1, Das D2, Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass; p. 360-365

[17] Niels Hempel & Ingolf Petrick & Frank Behrendt; Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production; p. 1409-1410