Employing indigenous microalgae for third generation biofuel production

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[*] Corresponding author, e-mail: thilini@uom.lk, Tel: +94712227964Abstract

ABSTRACT - Microalgae have been identified as a potential source for biofuel production by the researches. Cell disruption and chlorophyll removal of microalgae biomass are the most critical factors, which determine the lipid extraction yield and the biodiesel quality. Thus, the chlorophyll removal and cell disruption before lipid extraction is a mandatory step for the microalgae biodiesel production. This research investigated the growth rate and different pretreatment methods on lipid yield for selected microalgae species. *Desmodesmus sp., Scenedesmus sp.* & *Closteriopsis sp*. are the selected indigenous microalgae species, which were isolated from the Beira Lake, Colombo, Sri Lanka. *Desmodesmus sp* has shown the highest growth rate compared to other two species. Moreover, most suitable cell disruption method, which gave the highest lipid yield for each species were different.

Key words: Microalgae; cell disruption; biodiesel; growth rate

INTRODUCTION

The world is now focusing on third generation of biodiesel, where microalgae can be used for the production of biodiesel as a sustainable solution for the global energy crisis. Triacylglycerides, which serves as the energy storage in microalgae can be simply converted in to bio diesel by transesterification, after extracted. It has been discovered that the average lipid content of microalgae varies from 1%-70%of dry weight. (Kalana, 2018) Lipid content and lipid productivity of the micro algae vary with the type of the species (Khan, Rashmi, Hussain, Prasad & Banerjee, 2009)

Sampling, cultivation, biomass harvesting, pretreatment, cell disruption, chlorophyll removal, lipid extraction and trans-esterification of lipids are the directly involving steps in biodiesel production from microalgae. Cell disruption is a critical pretreatment step to maximize the product recovery in algae bio refinery process. High quality biodiesel can be produced from chlorophyll-reduced biomass (Li et al., 2016).

METHODOLOGY

Cultivation of microalgae

Desmodesmus sp, Scenedesmus sp and Closteriopsis sp. were cultivated in Bold's Basal Medium in photo bioreactor bottles, under cool white LED light at 5000lux, 12/12h light /dark photoperiod, at 30ºC. (Kurniawati, Mahajoeno, Sunarto & Sari, 2017). For each species, two separate experimental setups were prepared to determine the growth rate and to extract lipids with four weeks' time gap.

Measuring the growth rate

Samples were taken in particular time frequency and growth rates were measured by direct cell counting using hemi cytometer and measuring optical density using UV-vis spectrophotometer at 750nm wavelength. (Chirivella-Martorell, Briz-Redón & Serrano-Aroca, 2018)

Biomass harvesting and drying

Biomass was harvested using centrifugation at 25 °C and 7000rpm for 10minutes.Biomass pellets were separated and dried in an air-drying oven at 80^0C for 24hrs until a constant biomass weight is achieved.

Chlorophyll removal

Dried biomass of each strains was separated in to 80mg samples and chlorophyll removal was carried out for all samples except 2 samples from each

species. NaOH solution (1% w/v) and Ethanol were added in 4:1 v/v ratio.

Cell disruption

Chlorophyll removed biomass samples of three species were subjected to four different mechanical cell disruption methods such as Grinding (using a mortar and pestle), Autoclaving (121^0C, stationary phase for 20 minutes), Microwaving (900W & 2455MHz), and Water bath heating (20 minutes, at 90ºC). After pretreatments, samples (except grinding) were centrifuged at 7000rpm, 25ºC for 10minutes and dried at 80 ºC for 24 hours. (Onay, Sonmez, Oktem & Yucel, 2016)

Lipid extraction

Bligh & Dyer method was employed for the lipid extraction. Biomass pellets were resuspended in the distilled water, Methanol, and chloroform with the volume ratio of 9:10:10. Samples were homogenized by 30 seconds vortexing and resultant samples were centrifuged at 7000 rpm for 4 min at 25ºC. After, the created chloroform layer, which formed a dense layer at the bottom of the centrifuge tube, was carefully separated by using micropipette and they collected into the clean falcon tubes. (Taher, Al-Zuhair, Al-Marzouqi, Haik & Farid, 2014)

RESULTS AND DISCUSSION

Desmodesmus sp has a higher growth rate compared to other two species and *Scendesmus sp*. and *Closteropsis sp*. have nearly same growth rates according to results obtained after measuring optical density and direct cell count. All three species were tended to decrease their growth rate after 19 days of inoculation. End of the exponential growth phase is ideal for the harvesting of biomass for lipid extraction as identified by the literature. Presence of chlorophylls can decrease the transesterification efficiency and combustion efficiency of biodiesel. Therefore, chlorophylls were removed

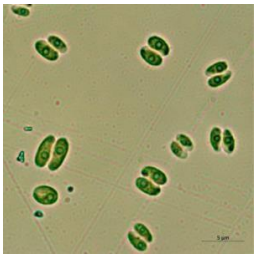

Figure 1: *Desmodesmus sp.*

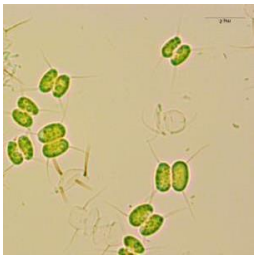

Figure 2: *Scenedesmus sp*.

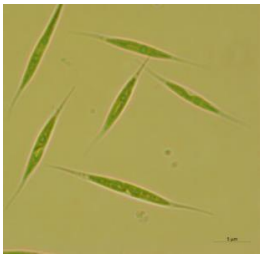

Figure 3: *Closteriopsis sp.*

before to the lipid extraction. Without any pretreatment method & non-removal of chlorophylls shown highest lipid yield for all three microalgae species. Doing pretreatments & removing of chlorophylls of microalgae will destroy the biomass & increase the quality of biodiesel.

The most efficient cell disruption methods for the three species were identified among selected cell disruption methods. As reported earlier dry biomass of microalgae contains about 5 -77 percentage of lipids, which has been found to vary in consistency from one species to another. Different cell disruption methods were practiced for the quantity of lipid extracted for the same strain in the study. The total lipid content of Scenedesmus sp. was found to vary depending upon different cell disruption methods adopted. The highest lipid yield of the *Scenedesmus sp*. was found as 39.29% by water bath method. As per these results, highest lipid yields 33% for *Desmodesmus sp* was obtained from Water bath which similar as *Scenedesmus sp.* and *Closteriopsis sp*

gives highest lipid yield as percentage of 37.72% from grinding.

CONCLUSION

One objective of present work was determining the growth rate of three microalgae species *Scenedesmus sp, Desmodesmus sp & Closteriopsis sp.* Bold Basal medium was the culture medium & microalgae were cultivated at room temperature with 7000lux of light intensity, 12:12 hours light: dark cycle. The microalgae strains of *Scenedesmus sp, Desmodesmus sp, Closteriopsis sp* were chosen because of their faster growth rate & higher lipid yield. *Desmodesmus sp* was observed the highest growth rate than other two species. This study has a comparative analysis of five physical pretreatment methods with removal & non-removal of chlorophylls for lipid yield from microalgae species. Water bath was observed as the best pretreatment method for lipid extraction for *Desmodesmus sp* & *Scenedesmus sp*. Results indicated that grinding method was best for lipid extraction of *Closteriopsis sp.*

REFERENCES

Chirivella-Martorell, J., Briz-Redón, Á., & Serrano- Aroca, Á. (2018). Modelling of Biomass Concentration, Multi-Wavelength Absorption and Discrimination Method for Seven Important Marine Microalgae Species. Energies, 11(5), 1089. doi: 10.3390/en11051089

Kalana, U. (2018). Assessment of The Growth Kinetics Of Selected Microalgae Species In Synthetic 016/j.biombioe.2014.02.034

Wastewater For The Production Of Biodiesel. Retrieved from http://dlib.pdn.ac.lk/handle/1/5757

Khan, S., Rashmi, Hussain, M., Prasad, S., & Banerjee, U. (2009). Prospects of biodiesel production from microalgae in India. Renewable And Sustainable Energy Reviews, 13(9), 2361-2372. doi: 10.1016/j.rser.2009.04.005

Kurniawati, F., Mahajoeno, E., Sunarto, & Sari, S. (2017). Effect of Light Intensity for Optimum Biomass and Lipid Production from Scenedesmus dimorphus (Turpin) Kützing. IOP Conference Series: Earth And Environmental Science, 75, 012012. doi: 10.1088/1755-1315/75/1/012012

Li, T., Xu, J., Wu, H., Wang, G., Dai, S., & Fan, J. et al. (2016). A Saponification Method for Chlorophyll Removal from Microalgae Biomass as Oil Feedstock. Marine Drugs, 14(9), 162. doi: 10.3390/md14090162

Onay, M., Sonmez, C., Oktem, H., & Yucel, M. (2016). Evaluation of Various Extraction Techniques for Efficient Lipid Recovery from Thermo-Resistant Microalgae - Comparison of Lipid Extraction Methods from Microalgae. American Journal of Analytical Chemistry, 07(02), 141-150. doi: 10.4236/ajac.2016.72012

Taher, H., Al-Zuhair, S., Al-Marzouqi, A., Haik, Y., & Farid, M. (2014). Effective extraction of microalgae lipids from wet biomass for biodiesel production. Biomass And Bioenergy, 66, 159-167. doi: 10.1