Improvement of Fatty Acids Composition of a Microalga Isolated from the Moroccan Seawater for Biodiesel Production

Salima Ouled Hajja
Geosciences, Water and Environment Laboratory (L-G2E) Faculty of Sciences
Mohammed V University (UM5) in Rabat
Rabat, Morocco
Email: fatiallalatserine@gmail.com

Miloudia Slaoui, Houria El Bakraoui
Energy, Materials and Sustainable Development (EMDD) Laboratory, Higher School of Technology-SALE, CERN2D
Mohammed V University (UM5) in Rabat
Rabat, Morocco
Emails: smslaoui2@gmail.com, houriya1234@gmail.com

Abstract— **The cellular biochemical composition of the isolated microalga Chlorella sp. was investigated in both favorable and nitrogen-depleted circumstances, with a particular focus on lipid classes and fatty acid distribution. When algal cells were grown in nitrogen-starvation media, the lipid and carbohydrate content increased, but the protein content decreased. Under control conditions, glycolipids were the most abundant lipid component at about 58.2% of total lipids, however under nitrogen stress conditions, neutral lipids became more prevalent at 74.8% of total lipids. The level of TAGs in nitrogen stressed cells was more than four times greater than in control cells. Oleic acid was the most prevalent fatty acid (47.2%) in the neutral lipids fraction. Under nitrogen stress, lipid quality analysis revealed that this alga has the potential to be used as a biodiesel feedstock.**

Keywords- Chlorella sp; biodiesel; stress conditions; Nitrogen; Phosphorus; bioenergy.

I. INTRODUCTION

Overuse of fossil fuels has resulted in an energy crisis and environmental issues [1]. Considering this, sustainable bioenergy, particularly biodiesel, has received a lot of attention in recent years. Biodiesel is a compound composed of fatty Acid Methyl Esters (FAME), created normally by the transesterification of vegetable oils or animal fats [1]. Microalgae are now seen as a viable feedstock for biodiesel production due to many benefits, including high photosynthetic efficiency, quick growth rate, and high lipid content [2]. In contrast to terrestrial energy crops, these organisms exhibit a significant lipid yield per unit of marginal land, possess the capability to harness sunlight and other nutrients to recycle carbon from fossil sources, and contribute positively to the environment [3]. The widespread commercial production of Microalgae biodiesel faces limitations due to various techno- logical and economic constraints [4]. Therefore, it is crucial to enhance both the yield of microalgal biomass and lipid production to maintain competitiveness and economic viability [5]. Various factors, including culture conditions like nitrogen and phosphorus stress, cultivation methods, temperature, light intensity, light/dark cycles, salinity, and pH, wastewater type can influence these parameters [6] – [8].

Chlorella has long been used commercially to make bioactive chemicals, animal feed, and nutrition for humans. Due to their high lipid output and environmental adaptability, reports of the potential of chlorella for the manufacture of biodiesel has increased in recent years [9]. There have been claims that several species of Chlorella might boost the lipid accumulation under conditions of nutritional restriction or famine. Given the preceding knowledge, it is critical to explore the buildup of lipids in microalgae growing under stress condition. In addition, total lipids are a useful tool for identifying oleaginous species and their fatty acid profiles offer a more targeted indication of the kind of substrate that is best for producing biodiesel.

In light of this, the purpose of this research is to assess the effects of nitrogen stress on biomass production, lipid accumulation, and fatty acid composition of an isolated microalga, Chlorella sp., cultured in f/2 medium.

II. METHODS

The strain Chlorella sp. investigated in this study was isolated from saltwater near Sidi Moussa beach in Morocco. Before being transferred to 100 mL beakers, the seawater samples were collected and filtered. Microalgae were identified using an optical microscope (Nikon ECLIPSE E200). Serial dilution was utilized to generate a pure culture. By inserting a loop of sample culture from the highest dilution tubes on the agar growth medium, a sterile loop was employed to form parallel streaks on the agar growth media. The petri dishes were covered with parafilm and incubated at 25◦C. After 30 day of incubation, f/2 medium has been employed in the maintenance of the culture of this species in 100 mL Erlenmeyer flask.

The cellular biochemical composition of the isolated microalga Chlorella sp. was investigated in both favorable and nitrogen-depleted circumstances, with a particular focus on lipid classes and fatty acid distribution after six days of cultivation. Carbohydrate concentration was determined by the phenol-sulfuric acid method. Total lipid extraction from dry biomass was carried out using the Bligh and Dyer technique. 100 milligrams of dry cells are maintained in 3.5 mL of a chloroform/methanol/water (2/1/0.5 v/v/v) mixture. The mixture was centrifuged for 15 minutes at 4000 rpm

 ISBN: 978-1-68558-089-6

after vertexing, and the organic phase was extracted and deposited in a tube that has been previously dried and weighed.

The lipid content was determined by using the following equation:

$$\text{Lipid content (\%)} = \text{ML/MA*100}$$

where ML (g) is the mass of the extracted lipids (corresponding to the difference in mass of the empty tubes and containing the dry lipids) and MA (g) is the mass of dry algal biomass. 500 μL of chloroform was used to resuspend the extracted lipids. 100 μL of this mixture was combined with 800 μL of 10% Boron trifluoride-methanol solution in a screw tube, and the combination was then heated to 100 ◦C in a water bath for 15 minutes. 750 μL of the solvent (100 μL of heptadecane in 10 mL of hexane) and 1.5 mL of water were added after cooling, and the mixture was vortexed for 2 minutes. The upper phase was retrieved with a Pasteur pipette, and 10 μL of it was injected into an Agilent gas chromatography (6850) system to characterize the methyl esters.

III. RESULTS AND DISCUSSION

Results indicate that, when algal cells were grown in nitrogen-starvation media, the lipid and carbohydrate content increased but the protein content decreased. Under control conditions, glycolipids were the most abundant lipid component about 58.2% of total lipids, however under nitrogen stress conditions, neutral lipids became more prevalent 74.8% of total lipids. The level of TAGs in nitrogen stressed cells was more than four times greater than in control cells. Oleic acid was the most prevalent fatty acid (47.2%) in the neutral lipids fraction. Similar trends have been documented in the cells of Chlorella vulgaris [10].

IV. CONCLUSION AND FUTURE WORK

The primary components of microalgal biomass consist of protein, carbohydrate, and lipid. Our findings demonstrated that when subjected to nitrogen deprivation, there was an elevation in carbohydrate and lipid content, accompanied by a reduction in protein content. Hence, environmental stress conditions play a crucial role in enhancing lipid quality for biodiesel production. Although nitrogen starvation can significantly increase the content of neutral lipids in microalgae, its main drawback is the limited biomass production.

Therefore, in the future work, it will be essential to further optimize the culture conditions for the isolated microalgae *Chlorella sp*. to achieve the desired quantity and quality of lipids for biodiesel production such as providing high light levels, limiting other nutrients.

REFERENCES

[1] J. Liu, J. Huang, Z. Sun, Y. Zhong, Y. Jiang and F. Chen, “Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic Chlorella zofingiensis: assessment of algal oils for biodiesel production,” Bioresource Technology, vol. 102, pp. 106–110, 2011.

[2] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins, “Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances,” The Plant. J, vol. 54, pp. 621–639, 2008.

[3] H. Li, “Environment-Enhancing Process for Algal Wastewater Treatment, Heavy Metal Control and Hydrothermal Biofuel Production: A Critical review”, Bioresource Technology, vol. 298, pp. 122 421, 2020.

[4] F. Chu, “Enhancing Lipid Production in Microalgae Chlorella PY-ZU1 with Phosphorus Excess and Nitrogen Starvation under 15% CO2 in a Continuous Two-Step Cultivation Process,” Chemical Engineering Journal, vol. 375, pp. 121912, 2019.

[5] K. R. Renjith, G. John, S. Muraleedharan Nair and N. Chandramohanakumar, “Biodiesel Prospective of Five Diatom Strains Using Growth Parameters and Fatty Acid Profiles”, Biofuels. 8, no.1, pp. 81-89, 2017.

[6] M. A. Yaakob, R. M. S. R Mohamed, A. Al-Gheethi, R. Gokare, and R. R. Ambati, “Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview”, Biomolecule, vol. 10, pp. 393, 2021.

[7] H. El. Bakraoui, M. Slaoui, D. Hmouni, and F. El aamri, “Impact of color of light and nitrogen concentration on Pavlova sp. biomass, cells size and biochemical composition,” Biofuels, 2022.

[8] H. EL Bakraoui, M. Slaoui, J. Mabrouki, D. Hmouni and C. Laroche “Recent Trends on Domestic, Agricultural and Industrial Wastewaters Treatment Using Microalgae Biorefinery System,” Applied Sciences, vol. 13, no.1, pp. 68, 2022.

[9] X. P. Zhou, L. Xia, H. M. Ge, D. L. Zhang, and C. X. Hu, “Feasibility of biodiesel production by microalgae Chlorella sp. (FACHB-1748) under outdoor conditions,” Bioresource. Technology, vol. 138, pp. 131-135, 2013.

[10] S. H. Ho, S. W. Huang, C. Y. Chen, T. Hasunuma, A. Kondo, C. Chang, and J. S, “Characterization and optimization of carbohydrate production from an indigenous microalga Chlorella vulgaris FSP-E” Bioresource. Technology, vol. 135, pp.157-165, 2013.

 ISBN: 978-1-68558-089-6