Microalgae Lipids: Exploring *Neochloris oleoabundans* for Applications in the Food Industry

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ABSTRACT

The microalgae are renowned for their ability to produce lipids abundantly, featuring fatty acid profiles that offer health benefits upon ingestion. These lipids emerge as strong contenders for enhancing global food security amidst the expansion of commodities and associated risk factors. Through the application of Design of Experiments (DOE) to pinpoint lipid accumulation of lipids from the microalgae *Neochloris oleoabundans*, researcher achieved a substantial increase from 6% to 30% in total lipids within the biomass, manipulating variables such as salinity, nitrogen concentration, light exposure, and sodium bicarbonate concentration. Thin-Layer Chromatography (TLC) served as a crucial tool for qualitatively identifying the extracted content, revealing the superior efficiency of hexane and methanol (5:1) extraction, as evidenced by observed spots.

Keywords: Microalgae. Microbial oil. *N. oleoabundans*. DOE. Lipid extraction.

1 INTRODUCTION

UN projections indicate that by 2050, the global population is projected to soar to 9.8 billion, presenting formidable challenges in ensuring food security sustainably. Notably, the demand for palm oil from Malaysia is anticipated to surge by 46%, exacerbating the urgency of addressing these challenges[1]. To address this issue, current research efforts are centered on enhancing the productivity of oilseed crops through genetic advancements. However, it is imperative to acknowledge the potential repercussions such as soil degradation and increased deforestation stemming from these endeavors.

Single Cell Oils[2,3], derived from microorganisms, emerge as a promising avenue for bolstering food security. Despite its roots dating back to the 19th century, the concept garnered significant attention in the 20th century, leading to extensive investigations into lipid accumulation by microorganisms. Microalgae, historically employed as food sources, exhibit particular promise owing to their remarkable oil productivity and adaptability to diverse environmental conditions, including freshwater, brackish, saline, and wastewater[4–6]. Their ability to thrive in such varied environments reduces cultivation costs through substrate reuse. Moreover, microalgae not only produce polyunsaturated fatty acids (PUFA) known for their health benefits but also demonstrate higher oil productivity compared to globally produced vegetable oils[7].

This study focuses on *Neochloris oleoabundans* UTEX #1185, a mixotrophic growth microalga initially isolated in the Rub' al-Khali desert in Saudi Arabia between 1958-1962. Previous observations by Bold and Chantanachat revealed substantial oil production within the cells after approximately three weeks of cultivation[8]. The primary objective of this research is to investigate the enhanced production of neutral lipids in *N. oleoabundans*, with BBM medium serving as a control and through the application of central composite rotatable design (CCRD) of the study aims to identify optimal parameters for lipid accumulation varying such as salinity, nitrogen concentration, light exposure, and sodium bicarbonate concentration.

2. MATERIAL & METHODS

2.1 BBM Cultivation and parameters of Design of Experiment.

Cells utilized as controls were cultivated in BBM medium[9], and the parameters modified for lipid accumulation included Sodium Bicarbonate (g/L), light intensity (µE m^{-2} s^{-1}), Nitrogen $NaNO_3$ (g/L), and Salinity NaCl (g/L)[10–13]. The levels of these parameters are detailed in Table 1 and were cultivated in 500mL Erlenmeyer flasks on a benchtop shaker (New Brunswick Classic C25KC Incubator Shaker), maintaining a photoperiod of 12/12 hours and a temperature of 30ºC for 28 days.

Table 1 A CCRD parameters for lipid accumulation in *N. oleoabundans* cultivation.

Level \ Factor	Light incidence (µE m^{-2} s^{-1})	Salinity NaCl (g/L)	Nitrogen NaNO3 (g/L)	Sodium bicarbonate $NaHCO_3$ (g/L)
-α	59	1,9	0,05	0,29
-1	100	5,0	0,15	0,50
0	200	12,5	0,30	1,00
1	300	20,0	0,50	1,50
α	341	23,1	0,55	1,71

2.2 Lipid extraction.

For lipid extraction, we employed a method involving biomass that had been previously dried in an oven at 30ºC for 24 hours, along with glass beads and a solution containing methanol:hexane (1:5). The extraction process began by adding methanol to the biomass in a Falcon tube, with a ratio of 0.5g of biomass to 5g of glass beads. The mixture was agitated for 30 minutes with intervals every 5 minutes. Subsequently, the remaining volume of hexane was added, followed by agitation for an additional 2 minutes. The Falcon tube was then centrifuged for 4 minutes at 5000g and the oil was recovered from the supernatant and concentrated using a rotary evaporator. This method ensures efficient extraction of lipids for further analysis and characterization.

2.3 Lipid profile by thin layer chromatography.

Following lipid extraction, was proceeded with the identification process using a two-step thin-layer chromatography (TLC) methodology on Silica gel chromatography plates (Silica gel on TLC Al foils Fluka Analytical). In the initial step, we employed a mobile phase composed of petroleum ether/diethyl ether/acetic acid (70/30/2) to facilitate the separation of degradation products originating from chlorophyll. Subsequently, in the second step, we utilized a mobile phase of petroleum ether/acetic acid (100/2), which enabled the separation of fatty acids, polar lipids, and non-polar lipids[14].

3 RESULTS & DISCUSSION

3.1 Cultivation for lipid production

The results demonstrated in Fig. 1 signalized that the parameters range tested didn't have critical values for lipid production that suggests the need for revised DOE with improved parameter ranges for lipid production. Despite this, this findings indicate a allowed to identify increase in lipid production compared to standard cultures, increasing from 6% of total lipids to 30%.

According to existing literature, modifications to the culture medium can induce abiotic stress, consequently triggering significant alterations in lipid accumulation within microorganisms. For instance, variations in light intensity can profoundly impact microalgae growth, contingent upon the saturation levels of photosynthetic apparatus; excessive light exposure may yield adverse effects, resulting in cellular damage. Light intensity can influence the growth of microalgae, depending on the possible saturation of each photosynthetic apparatus; excess light can yield contrary results, causing damage to the cell[15,16]. Therefore, the optimal range of light intensity for N. oleoabundans here was 188 µE m^{-2} s^{-1}. Besides, sodium bicarbonate fills in two purposes in reducing oxidative stress induced by photosynthesis and activating lipid production mechanisms, and providing a control against protozoan contamination[12,17]. Nonetheless, the observed increase in lipids associated with elevated NaCl concentrations is presumed to buoyancy in highly saline environments[18] but for this study the lowest concentration of salinity in the medium and with its interactions between the parameters would offer the best lipid accumulation. Among the various nutritional factors investigated, nitrogen emerges as a valued factor in improving lipid biosynthesis within microorganisms[19]. The literature suggests that restricting nitrogen availability in the culture medium augments the flux towards lipid biosynthesis pathways the critical value observed indicated a decrease to 0.35mM of nitrogen in the medium.

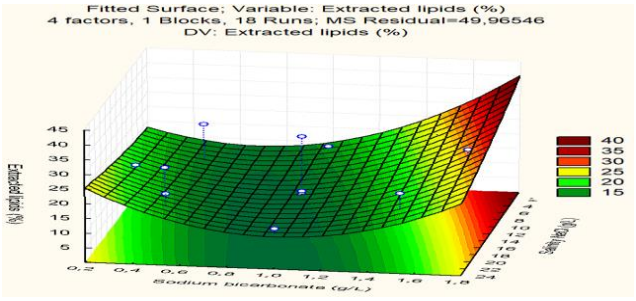

Figure 1 Adjusted Response Surface Graph of CCRD with nitrogen and light intensity parameters fixed at the central point.

3.2 Lipid extraction and identification.

The disorganization of algal biomass is necessary for lipid acquisition is a bottleneck because only the use of a solvent for extraction does not allow penetration through the rigid cell wall that protects the cell. Therefore, as observed in Figure 2, the methodology used was efficient in disrupting the entire biomass and conducting the extraction. Following extraction, Thin-Layer Chromatography (TLC) was employed for the identification of extracted components. By utilizing oleic acid as a standard for comparison with fatty acids and olive oil as a standard for triglycerides, comparison with the retention factor enabled the identification of effectively extracted triglycerides from the biomass, as illustrated in Figure 3. These results underscore the efficacy of extraction method in isolating target lipid components from the algal biomass, providing valuable insights into lipid acquisition processes.

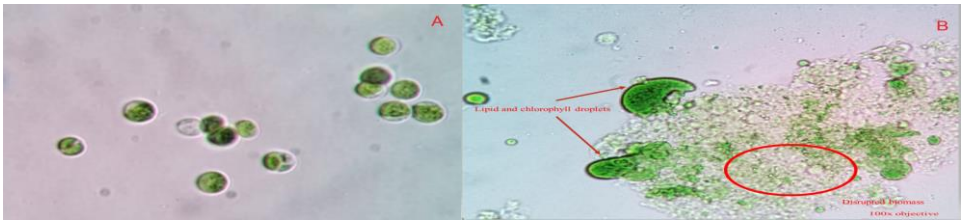

Figure 2 Optical microscopy with a 100x objective in the observation of the integrity of algal biomass before extraction (A) and after extraction (B) with the biomass completely disrupted.

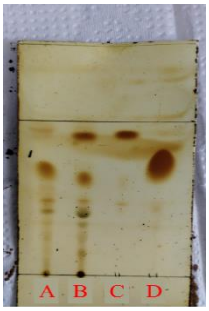

Figure 3 TLC of the lipid extracted by methodology outlined, and another involving only the use of hexane. The process occurred in two stages." 1º Mobile phase contendo Petroleum ether/diethyl ether/acetic acid 70/30/2 e a 2º Mobile phase Petroleum ether/acetic acid 100/2. Subtitle A – Sample extraction acting with hexane, 1mg diluted in 1.2 mL of chloroform; B – Sample extraction with hexane: metanol, 1mg diluted in 1.2 mL of chloroform; C – Olive oil (Standard); D – oleic acid (Standard).

4 CONCLUSION

The central composite rotatable design preliminarily allowed for an increase of 6% in lipid content to above 30% in some cases, while decreasing algal biomass production. The identification of lipids was possible through a comparison of sample retention times and the standards used. Correlations between the amount of lipids recovered from the detailed lipid extraction methodology and the Nile red and gravimetric methods will be employed to optimize the extraction of intracellular lipids.The critical values observed in the Central Composite Rotatable Design (CCRD) suggest the necessity for further experimentation to elucidate optimized parameters for lipid accumulation

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