Characterization of the Growth, Total Lipid and Fatty Acid Profiles in Microalga, *Nannochloropsis oceanica* under Different Nitrogen Sources

Majid Mahdieh*, Salimeh Shabani, and Mohammad Reza Amirjani

Department of Biology, Faculty of Science, Arak University, Arak 38156-8-8349, Iran

Received: January 10, 2018 / Revised: March 7, 2018 / Accepted: May 31, 2018

The properties of microalgae as bioresources for biodiesel production can be improved by adding nitrogen sources into the culture medium. Thus, *Nannochloropsis oceanica* CCAP 849/10 was cultured in f/2 media supplemented with five different forms of nitrogen at 0.88 mmol-N l⁻¹ each: ammonium bicarbonate (NH₄HCO₃), ammonium sulfate ((NH₄)₂SO₄), sodium nitrate (NaNO₃), ammonium nitrate (NH₄NO₃), and urea. The cell density, lipid content, and fatty acid profile of the microalgae were determined after 15 days of cultivation. The growth of *N. oceanica* based on cell number was lowest in the medium with NH₄NO₃ and increased significantly in the medium with NH₄HCO₃. Cells treated with (NH₄)₂SO₄ and NH₄NO₃ produced the highest total lipid contents (i.e., 65% and 62% by dry weight, respectively). The fatty acid profiles of the microalgae were significantly different in the various nitrogen sources. The major fatty acids detected in cultures supplemented with NH₄HCO₃, (NH₄)₂SO₄, NH₄NO₃, or urea were C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:5, and C22:6. However, the C16:1 content in the NaNO₃-supplemented culture was very low. This study highlights that the nitrogen source can strongly influence lipid production in *N. oceanica* and its fatty acid composition.

**Keywords:** Biodiesel, Chlorophyll a, nutrient, microalga, urea

**Introduction**

It seems that continual trust on energy of fossil fuel resources is labile, due to both reduction of world reserves and the emission of greenhouse gases connected with their application. Hence, there are strong studies aimed at replacing a renewable resource, containing potential biofuels, as energy sources. Recently, biodiesel fuel has received significant concern, because biodiesel is a biodegradable, reproducible and also non-toxic fuel. This fuel emits neither net carbon dioxide nor sulfur to the atmosphere and exhale less gaseous impu-
higher lipid contents and improvements to the quantity and quality of the lipids produced by microalgae are considered for the technology of biodiesel production from microalgae. Previous studies [9−11] have revealed that it is possible to manipulate the lipid yield and lipid properties of algal cells by the optimizing microalgae culture conditions (e.g. temperature and also light intensity) or features of nutrient medium (nitrogen, phosphates as well as iron concentrations). Microalgae biomass and biofuels production are changed by various physico-chemical factors such as nutrients, light intensity, temperature, pH and salinity [12, 13]. Especially between diverse nutritional agents, nitrogen is one of the most critical nutrients for algae growth, because this nutrient is a precipitant in all structural and also functional proteins such as enzymes, peptides, chlorophylls, energy transfer molecules, and genetic materials in algal cells [14, 15]. Wang et al. [16] reported that the nitrogen concentration in culture medium strongly affects both cell growth rate and cellular biochemical compositions in microalgae. In addition, numerous investigations have demonstrated that when the nitrogen is restricted in culture medium, microalgae decrease cell growth rate and raise their lipid or carbohydrate content, reducing protein synthesis [17].

Many microalgae species prefer ammonium, science less energy is needed for its assimilating into amino acids. In contrary, some microalgae such as Botryococcus braunii and Dunaliella tertiolecta prefer nitrate over ammonium for growth [18, 19]. Recent studies confirmed that some species of Chlorella also prefer nitrate rather than ammonium for growth, and these species also effectively use a variety of organic nitrogen sources such as urea, glycine, yeast extract (YE) and peptone [20, 21]. The results of Norici et al. [22] investigations proved that relevancy on the nitrogen source, biochemical composition can also be changed. For instance, protein content of Dunaliella salina was 2-times higher with ammonium than nitrate supplementation. In contrary, the lipid amount of Chlorella sorokiniana was over 2-folds more with ammonium than urea or nitrate supplementation [23]. Since the desirable source of nitrogen for growth varies from species to species, and the biochemical composition also can be differed by the supplemented nitrogen sources, it is essential to measure different nitrogen sources and take the most suitable source for each taxon in order to increase the efficiency of the goal product, like lipid and carbohydrate for biodiesel and bioethanol, respectively. Therefore, comprehension of the nitrogen sources effect on growth and also lipid value will ameliorate lipid yield and help large-scale commercial producers in selecting an appropriate fertilizer [24].

In this study, a oily microalga, Nannochloropsis oceanica, was selected for lipid production. The effects of various nitrogen forms such as nitrate, ammonium, and organic nitrogen (urea) on the cell growth, and the biochemical composition of N. oceanica were analyzed. Also, the microalgal lipid was converted to fatty acid methyl esters (FAME), and the fatty acid composition was assessed for measuring the effect on the resulted biodiesel properties.

Materials and Methods

Organism and culture treatments

Nannochloropsis oceanica CCAP 849/10 was purchased from the Culture Collection of Algae and Protozoa at Scotland. The microalga was grown in 500 ml flasks with f/2 medium [25] and continues aeration with air. N. oceanic was grown at 23 ± 1 °C and 100 µmol photons m−2 s−1 light intensity (L/D = 14:10). In order to study the effect of different nitrogen forms on the cell growth and biochemical composition of N. oceanica, sodium nitrate (NaNO3, 0.88 mmol N L−1) in F/2 medium was replaced by different nitrogen sources, including ammonium nitrate (NH4NO3), ammonium bicarbonate (NH4HCO3), ammonium sulfate (NH42SO4) and urea (CO(NH2)2). Initial nitrogen concentration was the same, at 0.88 mmol N L−1 and pH was adjusted to 8 after addition of each nitrogen source.

The cultures were grown for 15 days. The biomass, based on cell number and lipid quantity and quality of microalga were then assessed. All of the experiments were carried out in triplicate.

Cell density

The cell densities of the cultures were measured by hemacytometer cell counter.

The chlorophyll a content

The chlorophyll a (Chla) was determined spectropho-