Polyunsaturated fatty acids production utilizing five algae strains grown under autotrophic and mixotrophic conditions

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1. Introduction

Research on the development of algae mass production technologies has gained great biotechnological interest over the past years based on the ability of the specific organisms to bio-convert different organic macromolecules into valuable materials and renewable energy resources. Many algal strains are capable of growing under both autotrophic and mixotrophic nutrition. However, mixotrophic growth is more promising given the specific cultivation method utilizes organic substances and yields higher growth rates as compared to autotrophic conditions (Brennan and Owende 2010). Algae constitute an important carbon sink and cells often contain more than 50% of fats and oils, rich in ω-3, enabling the production of pharmaceuticals and raw material for biodiesel manufacture. These organisms yield various useful compounds such as pigments, vitamins and proteins (Abirami, Murugesan, and Narender 2016; Haznedaroglu et al. 2016).

Nutrition based on algae-derived fatty acids includes beneficial effects on human health, since eicosapentaenoic acid (EPA, 20:5 n3) and docosahexaenoic acid (DHA, 22:6 n3) constitute important ω-3 polyunsaturated fatty acids (PUFA). These molecules incorporate numerous nutraceutical and pharmaceutical applications. EPA and DHA comprise important agents for the treatment of cancer (D'Eliseo and Velotti 2016), cardiovascular diseases (Balk and Lichtenstein 2017) and hypertension (Colussi et al. 2016).

In this paper, we report a preliminary study for the development of a bioprocess for the production of algae rich in PUFA from biowaste. We present the results obtained from autotrophic growth of five microalgal strains while the presentation will also incorporate results from mixotrophic cultures conducted with the use of sugars.

2. Materials and methods

2.1. Microorganisms and growth conditions

Marine algae strains *Isochrysis galbana* 927/1, *Nannochloropsis oculata* 849/1, *Tetraselmis suecica* 66/4, *Microchloropsis gaditana* 849/5 and freshwater algae *Scenedesmus obliquus* 276/3A were obtained from the Culture Collection of Algae and Protozoa (CCAP). The medium used for the cultivation was f/2, apart from *Scenedesmus obliquus*, which was grown in Bold's Basal medium. Autotrophic growth was performed without addition of carbon source, whereas mixotrophic growth was conducted by supplementation of 1% glucose to the medium. The cultures were performed under batch conditions using 1L glass bottles with continuous shaking at 85 rpm and maintained under blue (450 nm) and red (650 nm) light (12 h light followed by 12 h darkness cycle) at room temperature. They were aerated with sterile air in the presence of CO_2 (≈5%), at a flow rate of 200 mL min^{-1}. All cultures were conducted in duplicate for each strain.

2.2. Analyses

Dry cell weight (DCW) and ash-free dry weight (AFDW)

DCW and AFDW was determined according to Borowitzka and Moheimani (2013). A known volume of algal culture was filtered through preweighed, precombusted glass-fiber filters (Whatman GF/C, 47 mm diameter) and washed with 10 mL of diluted HCl for freshwater strains or isotonic ammonium formate solution for saline strains. The filters were then subsequently dried at 100 °C for 1 h, cooled under vacuum overnight and weighed. The filters were then ashed in a muffle furnace at 450 °C for 5 h, cooled in a vacuum desiccator and weighed to obtain AFDW.

Optical density

Absorbance was measured using Jenway 7315 spectrophotometer in the range of 530-750 nm. *Isochrysis* spp. could not be measured through optical density measurements due to the formation of filaments.

2.3. Lipid extraction and quantification

Extraction of lipid was performed using the Folch method following modifications (Folch, Lees, and Sloane Stanley 1957). In brief, chloroform/methanol (2:1, v/v) was added to the sample and mixed. The mixture was centrifuged and the supernatant was transferred to a glass tube. Deionized water was added and mixed to allow phase separation. The methanol/water layer was removed and the chloroform phase containing the lipids was transferred to a pre-weighed

vial which was placed under a stream of N_2 gas. Following evaporation, the sample was maintained in a vacuum desiccator overnight and the vial was weighted.

3. Results and discussion

In autotrophic nutrition, *S. obliquus* was the fastest growing algae strain according to AFDW measurements and optical density (Figure 1). The strain produced the highest amount of total lipids and potentially ω-3 fatty acids (the complete profile of fatty acids will be included in the presentation). The total lipids content per AFDW was higher in *N. oculata*, while *T. suecica* was the second best producer in terms of total lipids content. Table 1 presents the algae biomass and lipid production of autotrophic nutrition.

Table 1: Algae biomass and lipid production under autotrophic growth

Algae Strain	Initial algae biomass ($g\ L^{-1}$)	AFDW ($g\ L^{-1}$)	Total lipids ($g\ L^{-1}$)	Total lipids (% of AFDW)
Tetraselmis suecica	0.063	1.02	0.8	78
Microchloropsis gaditana	0.063	0.66	-	-
Scenedesmus obliquus	0.063	2.39	1.33	56
Nannochloropsis oculata	0.063	0.59	0.65	110
Isochrysis galbana	0.063	0.38	0.05	13

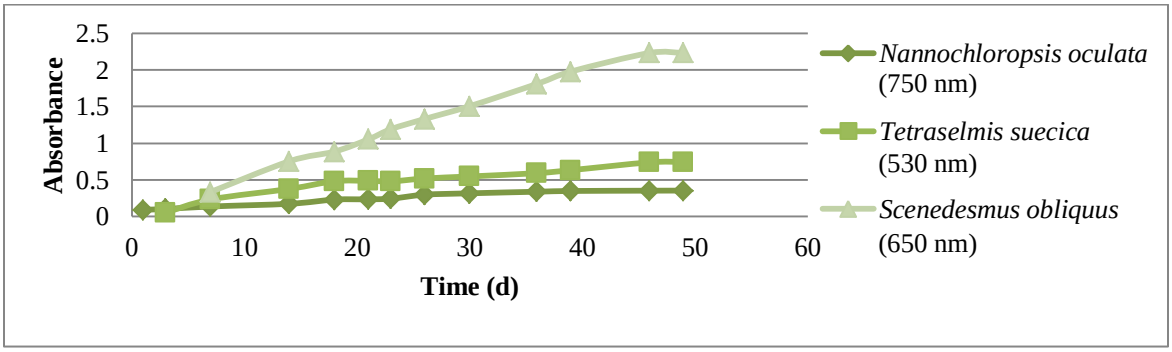

Figure 1: Algae strains growth under autotrophic conditions.

4. Conclusions

The presentation will include measurements of the AFDW and the total lipids of mixotrophic nutrition. Moreover, a comparison between the two cultivation methods will be given including the complete analysis of the total lipids analysis and the ω-3 fatty acids content for both autotrophic and mixotrophic cultures.

5. References

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