Enhancement of biomass and lipid production by *Isochrysis galbana* and *Scenedesmus obliquus* under mixo- and hetero-trophic growth

A. Nicodemou[1], M. Kallis[1], M. Koutinas[1]

[1]Department of l Chemical Engineering, Cyprus University of Technology, Limassol, 30 Archbishop Kyprianou Str., 3036, Cyprus
Keywords: *Scenedesmus obliquus, Isochrysis galbana*, microalgae, mixotrophic, lipid accumulation
Presenting author email: at.nicodemou@edu.cut.ac.cy

1. Introduction

The optimization of growth and added value commodities production by microalgae without substantial loss of biomass requires assessing the concentration of nutrients provided, such as carbon, nitrogen (N) and phosphorus (P), as a key factor to enhance the synthesis of bioactive compounds by the specific cells. *Scenedesmus obliquus* grown heterotrophically using glucose demonstrated elevated lipid accumulation, while the regulation of nitrogen and phosphorus was highly important for the conversion of the carbohydrate into fatty acids (Shen et al. 2020). Moreover, the use of different carbon sources could play a vital role on the production of microalgae biomass enhancing the manufacture of high-value products (Ma et al. 2021). The salinity level could increase the lipid content of the freshwater microalgae *Chlorella pyrenoidosa* (Yang et al. 2021), constituting salinity as a significant factor for enhancement of bioactive compounds synthesis, while the effect of light intensity and pH value on the growth and lipid production of *S. obliquus* hold the capacity to maximize the formation of lipids (Han et al. 2021).

The aim of this work is to enhance the cultivation of *Isochrysis galbana* and *S. obliquus* by testing different N and P concentrations, while evaluating microalgaes' growth, lipid productivity and organic carbon removal during mixotrophic growth. Furthermore, the aforementioned strains are preferred for wastewater treatment due to their capacity to perform high nutrients removal, growth rate and lipid productivity which can be used in the food and nutraceutical industries. *I. galbana* is a marine microalgae tolerant to phenolic compounds (Li et al. 2020) capable of growing under mixotrophic and heterotrophic conditions, while *S. obliquus* constitutes a freshwater microalgae demonstrating high photosynthetic capacity, fast growth and the ability to remove nutrients from wastewater (Song et al. 2021). Thus, a preliminary study will be additionally included assessing the cultivation of *I. galbana* and *S. obliquus* using as feedstock wastewater emitted from the olive industry.

2. Materials and methods

2.1. Microorganisms and growth conditions

The marine algae *I. galbana* CCAP927/1 and the freshwater strain *S. obliquus* CCAP276/3A were cultivated in f/2 and Bolds Bassal medium respectively. Autotrophic growth was performed without addition of organic carbon source, whereas mixotrophic cultivation was conducted by supplementing the medium with 1% D-glucose. Cultures were performed under batch conditions using 1 L glass bottles with 800 mL working volume under continuous shaking at 100 rpm and room temperature. Autotrophic and mixotrophic cultures were maintained under blue (450 nm), red (650 nm) and white fluorescent light (12 h light followed by 12 h darkness cycle) at 100 μmol s^{-1} m^{-2} of light intensity. The flasks were aerated using sterile air and 350 mL min^{-1} flow rate, in the presence of CO_2 (approximately 5%). All cultures were conducted in duplicate for each strain.

2.2. Analyses

Dry cell weight and ash-free dry weight was determined according to Borowitzka and Moheimani (2013). Absorbance was determined using a Jenway 7315 spectrophotometer at 650 nm. The content of reducing sugars was obtained employing the 3,5-dinitrosalicilic acid (DNS) assay (Miller 1959), while the extraction of lipids was performed via the Folch method (Folch et al. 1957). Lipid transesterification with BF_3 and analysis of fatty acid composition were performed as described by Araujo et al. (2008).

3. Results and Discussion

The enhancement of *I. galbana* and *S. obliquus* cultivation was assessed by exploring different concentrations of N and P during mixotrophic growth. The results indicate that neither strain could grow under 0 ppm of N and P (Table 1). Moreover, there was no significant differences on biomass production by *I. galbana* observed between the use of 20, 40 and 80 ppm N and 5, 10 and 15 ppm P ($p \geq 0.05$). The aforementioned strain consumed the highest content of glucose (reducing the initial 10 g/L supplied by 85%) employing media containing 40 ppm N, while the different concentrations of P applied resulted in similar glucose consumption (51-67%). The highest biomass production by *S. obliquus* was achieved using 50 and 100 ppm N, where no significant differences on biomass formation were observed. As far as the use of different P concentrations is concerned, media employing 20, 40 and 80 ppm P resulted in the highest biomass content as confirmed by statistical analysis. *S. obliquus* grown under 100 ppm N consumed the whole content of glucose supplemented (100%), while the application of 80 ppm P resulted in the highest glucose assimilation (89%) performed by the specific strain.

Table 1 Biomass production, lipid productivity and glucose removal performed under different N and P concentrations by *I. galbana* and *S. obliquus*.

	N (ppm)	Biomass (g/L/d)	Lipids (mg/L/d)	Glucose removal (%)
S. obliquus	0	0	9.5	12.5
	12.5	0.04	23.2	25.3
	25	0.05	19.0	18.1
	50	0.16	22.1	73.9
	100	0.16	34.1	100.0
I. galbana	0	0.02	11.8	36.0
	20	0.14	35.7	62.0
	40	0.20	39.3	84.8
	80	0.18	32.5	76.6
	P (ppm)			
S. obliquus	0	0.07	20.3	15.2
	20	0.13	25.7	62.6
	40	0.15	23.8	76.3
	80	0.13	26.9	89.1
I. galbana	0	0.03	13.7	64.2
	5	0.10	22.5	50.9
	10	0.09	20.5	67.1
	15	0.10	21.7	53.5

4. Conclusions

The present work demonstrated that different bioprocess parameters are important to enhance the production of lipids from *I. galbana* and *S. obliquus* during mixotrophic growth. The presentation will include the cultivation of the two microorganisms employing wastewater from the olive industry, comparing autotrophic and mixotrophic cultivation, which will include complete analysis of the ω-3 fatty acids content of both trophic modes. The study provides valuable insight relevant to the use of *I. galbana* and *S. obliquus* for the development of advanced algal biorefinery approaches.

5. References

Araujo, Pedro, Thu Thao Nguyen, Livar Frøyland, Jingdong Wang, and Jing X. Kang. 2008. "Evaluation of a Rapid Method for the Quantitative Analysis of Fatty Acids in Various Matrices." *Journal of Chromatography A* 1212 (1–2): 106–13. https://doi.org/10.1016/j.chroma.2008.10.006.

Borowitzka, Michael A., and Navid R. Moheimani. 2013. *Algae for Biofuels and Energy*. *Journal of Chemical Information and Modeling*. Vol. 53. Springer. https://doi.org/10.1017/CBO9781107415324.004.

Folch, Jordi, M Lees, and G.H Sloane Stanley. 1957. "A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues." *J. Biol. Chem*, no. 226: 497–509.

Han, Wei, Wenbiao Jin, Ze Li, Yubin Wei, Zhongqi He, Chuan Chen, Changlei Qin, Yidi Chen, Renjie Tu, and Xu Zhou. 2021. "Cultivation of Microalgae for Lipid Production Using Municipal Wastewater." *Process Safety and Environmental Protection* 155: 155–65. https://doi.org/10.1016/j.psep.2021.09.014.

Li, Hao, Fanping Meng, Yuejie Wang, and Yufei Lin. 2020. "Removal of Phenol by Isochrysis Galbana in Seawater under Varying Temperature and Light Intensity." *Journal of Oceanology and Limnology* 38 (3): 773–82. https://doi.org/10.1007/s00343-019-9125-6.

Ma, Xiangmeng, Yuwei Mi, Chen Zhao, and Qun Wei. 2021. "A Comprehensive Review on Carbon Source Effect of Microalgae Lipid Accumulation for Biofuel Production." *Science of The Total Environment* 806: 151387. https://doi.org/10.1016/j.scitotenv.2021.151387.

Miller, Gail Lorenz. 1959. "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar." *Analytical Chemistry* 31 (3): 426–28. https://doi.org/10.1021/ac60147a030.

Shen, Xiao Fei, Lin Jun Gao, Shou Biao Zhou, Jia Le Huang, Chen Zhi Wu, Qi Wen Qin, and Raymond Jianxiong Zeng. 2020. "High Fatty Acid Productivity from Scenedesmus Obliquus in Heterotrophic Cultivation with Glucose and Soybean Processing Wastewater via Nitrogen and Phosphorus Regulation." *Science of the Total Environment* 708: 134596. https://doi.org/10.1016/j.scitotenv.2019.134596.

Song, Yanan, Xiaodan Wang, Hongli Cui, Chunli Ji, Jinai Xue, Xiaoyun Jia, Ruiyan Ma, and Runzhi Li. 2021. "Enhancing Growth and Oil Accumulation of a Palmitoleic Acid–Rich Scenedesmus Obliquus in Mixotrophic Cultivation with Acetate and Its Potential for Ammonium-Containing Wastewater Purification and Biodiesel Production." *Journal of Environmental Management* 297 (January): 113273. https://doi.org/10.1016/j.jenvman.2021.113273.

Yang, Zi-yan, Feng Gao, Jun-zhi Liu, Jin-sheng Yang, Mei Liu, and Ya-ming Ge. 2021. "Bioresource Technology Improving Sedimentation and Lipid Production of Microalgae in the Photobioreactor Using Saline Wastewater." *Bioresource Technology*, no. November: 126392. https://doi.org/10.1016/j.biortech.2021.126392.