CYPRUS UNIVERSITY OF TECHNOLOGY

Environmental Bioprocessing Laboratory
Department of Chemical Engineering
30 Archbishop Kyprianou Str.
3036
Limassol
Cyprus

Cyprus University of Technology

Environmental Bioprocessing Laboratory

Enhancement of biomass and lipid production by *Isochrysis galbana* and *Scenedesmus obliquus* under mixotrophic growth

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

[1]Department of Chemical Engineering, Cyprus University of Technology, 30 Archbishop Kyprianou Str., 3036, Limassol, Cyprus

*andonianicodemou@hotmail.com

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 nitrogen (N) and phosphorus (P). 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 [1].

Isochrysis galbana and *Scenedesmus obliquus* 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 [2] 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 [3].

Aim and Objectives

The main objectives of the present work comprise:

- ❑ Enhancement of the cultivation of *I. galbana* and *S. obliquus* by testing different N and P concentrations and organic carbon source in the medium, while evaluating microalgae's growth, lipid productivity and organic carbon removal during mixotrophic growth.
- ❑ Characterization of two types of biowaste derived from olive industry: table olive wastewater (TOW) and olive pomace (OP).
- ❑ The development of algal biorefinery concept employing *I. galbana* and *S. obliquus* for the treatment of TOW and OP, respectively.

Materials and methods

The marine algae strain *I. galbana* was cultivated using f/2 medium, while *S. obliquus* was grown in Bold's Basal medium. Limitations/deficiencies of nitrate and phosphate in the medium were monitored by adjusting $NaNO_3$, $NaH_2PO_4.2H_2O$, K_2HPO_4 and KH_2PO_4 concentration. Mixotrophy was conducted by supplementation of 1% D-glucose or lactose to the medium. Flasks were performed under batch conditions using shaking at 100 rpm and room temperature. Cultures were maintained under blue and red light (12:12 h ligh:dark cycle) at 100 μmol s^{-1} m^{-2} light intensity while aerated using sterile air in the presence of CO_2 (≈5%).

Analyses:

- The growth of each culture was monitored by measuring the ash-free dry weight (AFDW) and optical density.
- Reducing sugars in mixotrophic algae cultures and wastewaters were determined by the DNS method.
- Lipids were extracted from algae cells and OP using the Folch method.
- The fatty acid profile of lipids was determined as fatty acid methyl esters by gas chromatography, following conversion of fatty acids to methyl esters with BF_3 in methanol.
- Polyphenol concentration was determined using Folin-Ciocalteu method.
- Phosphorus concentration was assessed by colorimetric ascorbic acid method.
- Determination of cellulose, hemicellulose and lignin compounds concentration was performed by employing a Fibre-Bag System.
- Nitrate nitrogen NO_3-N in wastewaters was determined using NANOCOLOR Nitrate 50 kit.
- pH, conductivity and salinity were measured using EC400 ExStik ®.

Results and Discussion

Organic carbon source

In mixotrophic cultivation, 1% D-glucose and 1% lactose were separately employed as organic carbon source for biomass and lipid production. Lactose comprises a disaccharide composed of galactose and glucose comprising the main constituent of whey permeate (WP), which is an important bioresource for valorization in biowaste-based biorefineries.

- *I. galbana* exhibited low biomass productivity using lactose mixotrophically.
- *S. obliquus* showed high growth rate (0.15 g L^{-1} d^{-1}) and lipid assimilation (19% of AFDW).
- Lactose consumption by *S. obliquus* remained at 33.3%.
- Mixotrophic cultivation employing glucose resulted in higher lipids as percentage of AFDW for *S. obliquus* (21.2% using glucose over 19.0% using lactose).

Table 1: Overview of biomass production, lipid productivity and organic carbon removal under autotrophic and mixotrophic nutrition employing glucose and lactose

Genus/ species	Carbon source	Biomass productivity (g L^{-1} d^{-1})	Lipids (% AFDW)	Lipid productivity (mg L^{-1} d^{-1})	Organic carbon removal (%)
I. galbana	CO_2	0.033	16.6	6.18	-
	Glucose	0.057	22.0	24.85	55.9
	Lactose	0.029	13.8	4.0	16.4
S. obliquus	CO_2	0.030	13.0	4.0	-
	Glucose	0.13	21.2	21.97	95.6
	Lactose	0.15	19.0	30.61	33.3

Based on the results of Table 1, glucose was selected as organic carbon source in subsequent experiments targeting the enhancement of biomass and lipid productivity.

N and P concentration

The N and P concentrations applied comprised 40 and 53.1 ppm respectively, as per the Bold's Basal medium, as well as 12 and 1.1 ppm, as per the f/2 media respectively.

Table 2 Biomass production, lipid productivity and glucose removal performed under different N and P concentrations by *I. galbana* and *S. obliquus.*

	N (ppm)	Biomass conc. (g L^{-1})	Lipids productivity (mg L^{-1} d^{-1})	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

N and P concentration

- Neither strain could grow under 0 ppm of N and P.
- Significant differences on biomass production by *I. galbana* were not observed using 20, 40 and 80 ppm N as well as 5, 10 and 15 ppm P ($p \geq 0.05$).
- *I galbana* consumed the highest content of glucose employing media containing 40 ppm N. Also, 10 ppm P achieved the highest glucose reduction by 67.1%.
- Highest biomass production by *S. obliquus* was achieved using 50 and 100 ppm N ($p \geq 0.05$).
- *S. obliquus* grown under 100 ppm N consumed the total content of glucose supplemented (100%), while the application of 80 ppm P resulted in the highest glucose assimilation (89%) achieved.

Fatty acid biosynthesis by *I. galbana* and *S. obliquus* was assessed under various N and P contents as shown in Fig. 1.

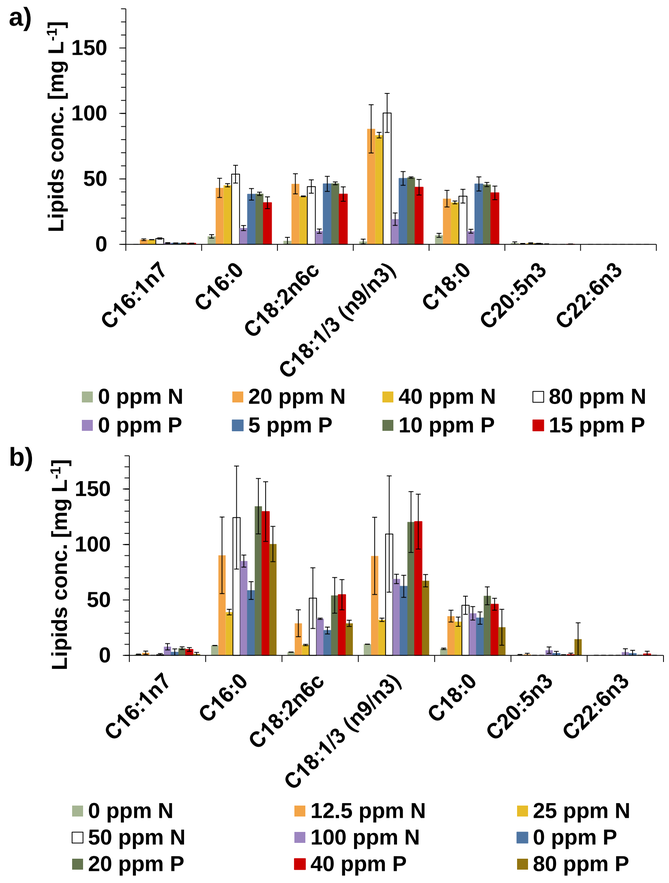

Fig.1 Fatty acid profiles obtained by (a) *I. galbana* and (b) *S. obliquus* cultivated under different N and P concentrations.

- ❑Significant differences were not observed in the fatty acid composition by *I. galbana* using 20, 40 and 80 ppm N as well as 5, 10 and 15 ppm P.
- ❑Significant differences were not observed for *S. obliquus* using 12.5, 50 and 100 ppm N. Although 80 ppm P for *S. obliquus* produced lower amounts of fatty acids, the specific content didn't affect C16:0, C16:1n7 and C18:0 production.

Biowaste characterization

OP constitutes the solid residue obtained following olive oil extraction, in large quantities. **TOW** is generated by the treatment of olives required to become edible. TOW used in this study was collected every 2 d over a 12 d period for the initial washing step with water (TOW WS) and every 1 week over a three-week period for the fermentation step conducted using 10% NaCl. The process applied to produce each effluent comprised mixing 1.5 kg of black olives with 3 kg of water or brine.

Table 3: Physicochemical characterization of OP and TOW

	OP	TOW WS	TOW FS
pH	4.9	5.5-6.6	5.4-6.7
Conductivity (mS/cm)	2.6	1.0-1.5	109.2-128.1
Salinity (ppt)	1.3	0.5-0.8	63.6-70.4
Extractives r.t. (% DW)	11.9	-	-
Ash (% DW)	4.6	-	-
Lignin (% DW)	37.2	-	-
Hemicellulose (% DW)	11.1	-	-
Cellulose (% DW)	25.8	-	-
NO_3-N	0.1 % DW	0.0-7.3 mg/L	5.3-15.7 mg/L
PO_4-P	0.04 % DW	1.4-14.6 mg/L	7.9-37.7 mg/L
Sugars	6.0 % DW	0.0-0.2 g/L	0.3-0.5 g/L
Polyphenols	0.6 % DW	0.0-171.6 mg/L	170.8-772.9 mg/L
Oil (% DW)	12.5	-	-

Based on the results of Table 3, OP consists of lignocellulosic matrix, polyphenols, soluble sugars and oil. Also, the high phenolic content and salinity of TOW constitutes the effluent difficult to treat via biological methods. Fig. 2 presents a simplified process flow diagram for the biorefinery based on (a) OP and (b) TOW with the use of microalgae.

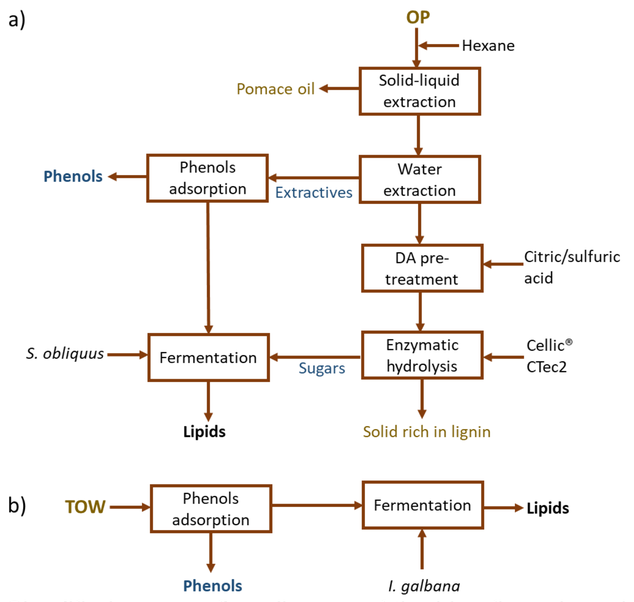

Fig.2 Simplified process flow diagram of the biorefinery based on (a) OP and (b) TOW.

References

1. Nicodemou, A. et al. (2022), Water, 14(2), pp. 1–14.
2. Li, H. et al. (2020), Journal of Oceanology and Limnology, 38(3), pp. 773–782.
3. Song, Y. et al. (2021), Journal of Environmental Management. 297(January), p. 113273.

CONCLUDING REMARKS

- Optimization of algae cultures is necessary to enhance the production of biomass and lipids, as well as the consumption of organic carbon.
- Results on the effects of N and P concentration in cultures suggest 100 ppm N and 40 ppm P as the optimal concentrations for *S. obliquus* as well as 40 ppm N and 10 ppm P for *I. galbana.*
- Based on physicochemical characterization, OP and TOW constitute excellent candidates for the development of a closed-loop biorefinery through use of algal biomass following lipids extraction.

FUTURE WORK

- ➢ Future work involves the fractionation of OP into its main constituents. Glucose will be recovered from the cellulose fraction and xylose from the hemicellulose fraction.
- ➢ Isolation of polyphenols from water extract of OP and TOW will be explored using different resins aiming to commercialize the molecules extracted in the food and cosmetic industry.