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Biomass and lipid production utilizing five algae strains grown under autotrophic, mixotrophic and heterotrophic conditions

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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 [1]. Microalgae can be cultivated autotrophically, heterotrophically and mixotrophically based on their ability to adapt to specific environmental conditions [2]. However, mixotrophic growth is more promising given that the specific cultivation method utilizes organic substances and yields higher growth rates as compared to autotrophic conditions [3]. Algae constitute an important carbon sink holding multiple applications in the pharmaceutical, nutraceutical and food industries, based on their capacity to produce various useful compounds such as ω-3 fatty acids, pigments, vitamins and proteins [4].

Aim and Objectives

The main objectives of the present work comprise:

- ❑ Evaluation of biomass and lipid productivity of five important microalgae strains *Isochrysis galbana, Microchloropsis gaditana, Scenedesmus obliquus, Nannochloropsis oculata* and *Tetraselmis suecica* under autotrophic, mixotrophic and heterotrophic conditions.
- ❑ Selection of the most effective strains for future application in algae biorefineries.

Materials and methods

Microorganisms and growth conditions:
Marine algae strains *Isochrysis galbana, Nannochloropsis oculata, Tetraselmis suecica* and *Microchloropsis gaditana* were cultivated using f/2 medium, while the freshwater algae *Scenedesmus obliquus* was grown in Bold's Basal medium. Mixotrophic and heterotrophic growth was conducted by supplementation of 1% D-glucose to the medium. The cultures were performed under batch conditions with shaking at 100 rpm and room temperature. Autotrophic and mixotrophic cultures were maintained under blue and red light (12:12 h ligh:dark cycle) at 30 μmol s^{-1} m^{-2} light intensity. Flasks were 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
- Total sugars in mixotrophic and heterotrophic algae cultures were determined by the DNS method
- Lipids were extracted and quantified with chloroform/methanol 2:1 (v/v) using the Folch method
- The fatty acid profile of lipids was determined as fatty acid methyl esters (FAME) by gas chromatography, following conversion of fatty acids to methyl esters with boron trifluoride in methanol

Results and Discussion

Algal biomass production

Autotrophic nutrition produced the lowest content of biomass for all microalgae strains, except from *M. gaditana*, where heterotrophy was the least productive trophic mode (Fig. 1).

- *S. obliquus* included the highest ash free dry weight in all trophic conditions producing 1.26 g L^{-1} under autotrophic, 3.16 g L^{-1} under mixotrophic and 2.92 g L^{-1} in heterotrophic culture conditions.
- *I. galbana, M. gaditana*, *S. obliquus* and *T. suecica* resulted in higher biomass production in mixotrophy as compared to autotrophic and heterotrophic nutrition, while *N. oculata* performed higher biomass production in heterotrophy.

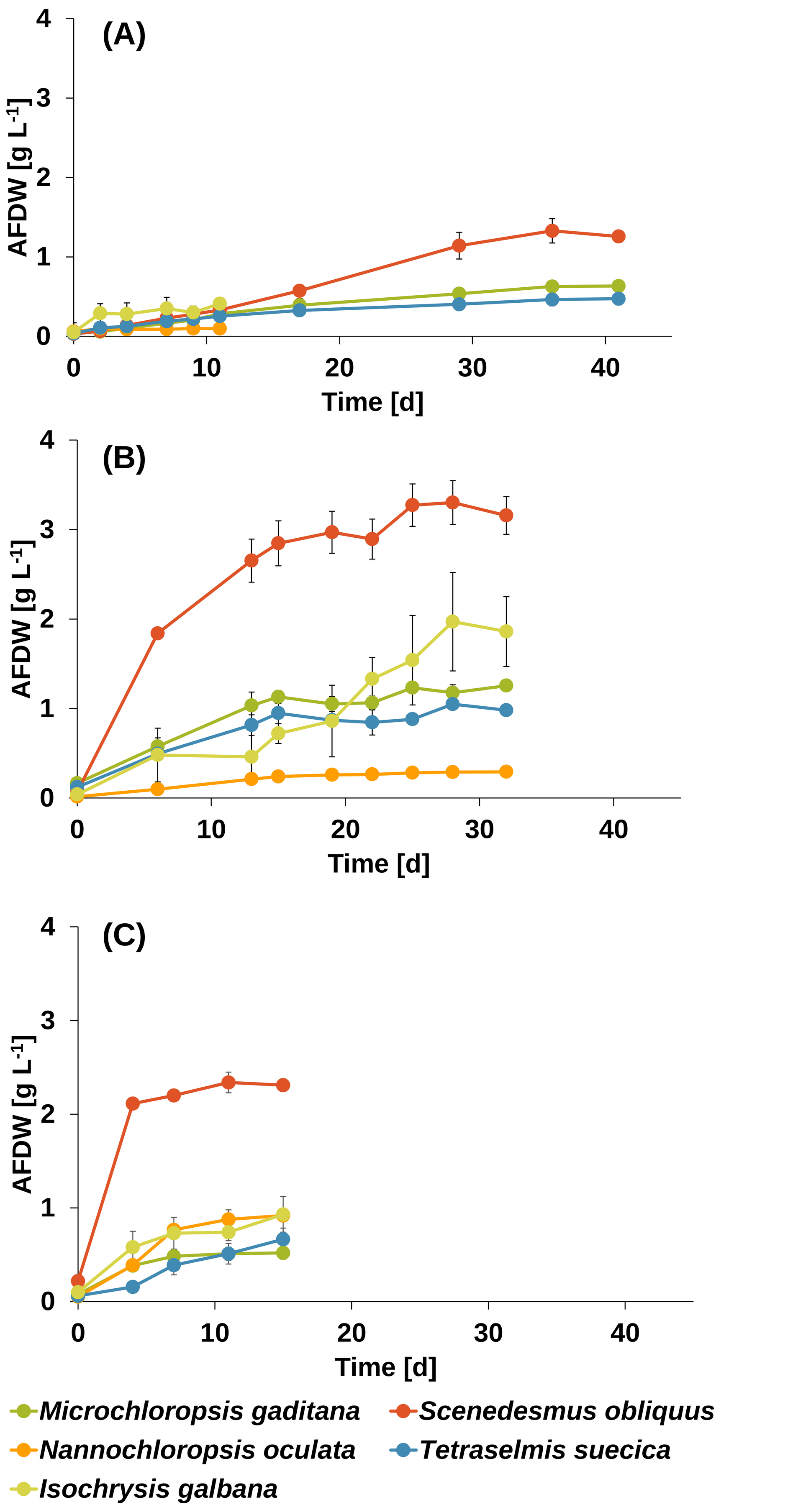

Fig. 1: Algae biomass growth using (A) autotrophic, (B) mixotrophic and (C) heterotrophic nutrition

Lipid content and productivity

Fig. 2 illustrates the total lipids extracted from the five microalgae strains evaluated in different trophic conditions.

- *Nannochloropsis oculata* performed the highest lipid accumulation that reached 44.5% of AFDW in autotrophy and 36.9% of AFDW in mixotrophy. However, *Nannochloropsis oculata* was the least productive strain in heterotrophy exhibiting lipid content of 9.2%.
- *Microchloropsis gaditana* accumulates higher lipid content in autotrophic nutrition (26.5% of AFDW) followed by heterotrophy (19% of AFDW).
- *Scenedesmus obliquus* produced more lipids in mixotrophy (21.2% of AFDW) rather than autotrophic and mixotrophic nutrition which are similar in lipid production (13.0% and 14.8% of AFDW, respectively).
- *Tetraselmis suecica* produced 28.5% lipids of AFDW in autotrophy. Mixotrophic and heterotrophic nutrition produced lowest amounts of lipids.
- *Isochrysis galbana* in mixotrophic nutrition included the highest lipid productivity of 24.85 mg L^{-1} d^{-1}.

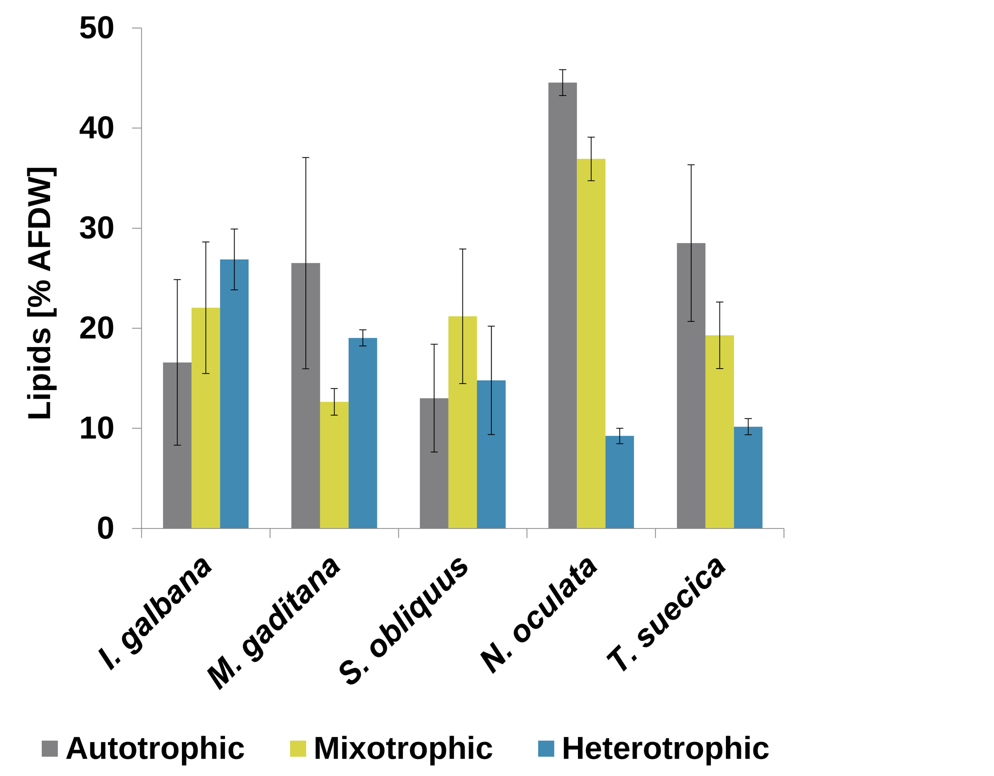

Fig. 2: Lipid production (% of AFDW) under autotrophic, mixotrophic and heterotrophic growth

Carbohydrate concentration

In mixotrophy and heterotrophy, the culture medium was enriched with 1% D-glucose as organic carbon source. The results showed that during the whole-time course of mixotrophic and heterotrophic growth, *Scenedesmus obliquus* and *Isochrysis galbana* consumed significant quantities of glucose.

- ❑ *Scenedesmus obliquus* consumed 95.6% and 55.4% of the initial glucose concentration in mixotrophic and heterotrophic nutrition, respectively.
- ❑ *Isochrysis galbana* used 55.9% glucose in mixotrophy, while 57% was utilised during the dark heterotrophic growth conditions.

Fatty acid composition

All algae strains exhibited production of similar classes and proportions of fatty acids (Table 1). The most abundant saturated acid was palmitic acid C16:0, while C18:1n9c/C18:3n3 was the most abundant PUFAs.

- *Isochrysis galbana* was unique in producing a high content of linoleic acid C18:2n6c under heterotrophic nutrition (87.9 mg g^{-1} of AFDW). Autotrophic and mixotrophic conditions produced lower amounts of fatty acids, since these trophic modes formed lower amounts of lipids (% of AFDW) for this microorganism.
- *Microchloropsis gaditana* produced large quantities of C18:1n9c/C18:3n3 (108.6 mg g^{-1} of AFDW) in autotrophy. Mixotrophy accumulated the lowest amounts for almost all fatty acids detected for this strain, except from linoleic acid and stearic acid C18:0.
- Palmitic acid was the most abundant saturated fatty acid detected in *Scenedesmus obliquus* comprising 61.2 mg g^{-1} of AFDW under mixotrophic nutrition. Results show that this microorganism produce higher amounts of fatty acids under mixotrophic conditions.
- *Tetraselmis suecica* also performed high contents of C18:1n9c/C18:3n3 (22.3-99.6 mg g^{-1} of AFDW).

Table 1: Fatty acid profile of the microalgae strains tested. Data reported as mg g^{-1} of dry weight (A: autotrophy, M: mixotrophy, H: heterotrophy)

		C16:0	C16:1	C18:0	C18:1/ C18:3	C18:2	C20:5	C22:6
I. galbana	A	18.0	0.0	12.9	14.0	11.4	0.0	0.0
	M	44.6	2.0	28.5	65.0	38.1	0.5	0.0
	H	74.0	2.5	61.9	77.4	87.9	1.3	0.4
M. gaditana	A	68.6	1.9	7.9	108.6	9.9	8.1	0.0
	M	22.9	1.1	8.2	9.1	20.6	0.6	0.0
	H	63.7	3.5	33.4	66.9	51.1	0.0	0.0
S. obliquus	A	30.8	0.0	10.6	30.3	2.8	0.0	0.0
	M	61.2	3.6	12.5	57.7	27.5	1.2	0.0
	H	23.1	0.6	10.9	17.1	10.1	1.1	0.0
N. oculata	A	70.8	0.0	45.3	31.8	24.8	0.0	4.8
	M	61.3	3.5	23.7	48.3	28.6	2.2	2.3
	H	31.2	0.5	15.4	28.6	18.3	0.0	0.5
T. suecica	A	66.5	1.1	10.1	99.6	11.7	9.2	0.0
	M	43.2	2.3	19.9	42.4	22.9	1.0	0.0
	H	22.6	1.4	11.7	22.3	15.0	1.1	0.0

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CONCLUDING REMARKS

- *Scenedesmus obliquus* and *Isochrysis galbana* were selected for further investigation via consideration of biomass and lipid production, as well as the consumption of large quantities of organic carbon that enable the development of algae biorefineries.
- *Scenedesmus obliquus* in heterotrophy included the highest biomass productivity (0.14 g L^{-1} d^{-1}).
- *Isochrysis galbana* in mixotrophic nutrition performed the highest lipid productivity of 24.85 mg L^{-1} d^{-1}.

FUTURE WORK

- ➢ Optimization of culture conditions for the selected microalgae strains: Nitrate and phosphate concentration, light intensity and organic carbon source.
- ➢ Explore the use of *Scenedesmus obliquus* and *Isochrysis galbana* for the development of a closed-loop biorefinery through use of algal biomass following lipids extraction.