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Carbon, nitrogen, and phosphorus removal, and lipid production by three saline microalgae grown in synthetic wastewater irradiated with different photon fluxes

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ABSTRACT

Mariculture production has increased in the last decades, with untreated wastewater discharged directly into the sea, impacting coastal ecosystems. There is a need for mariculture wastewater treatment systems that are cost-effective. This can be met by the implementation of wastewater treatment systems that in addition to removing pollutants are capable of producing valuable by-products such as biomass for the biofuel industry. In this study, *Dunaliella* sp., *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae were cultivated in controlled environments simulating mariculture wastewaters. Single stage culture systems were used to grow these microalgae, the growing conditions included inducing stress with different photon flux densities (900, 1500 and 2000 $\mu mol\, m^{-2}\, s^{-1}$), and low carbon, nitrogen and phosphorus concentrations obtained at the stationary phase, in order to force these microalgae to increase their lipid content. The best results were obtained with *Tetraselmis* sp., which achieved 132.8 $mg\, L^{-1}\, day^{-1}$ of biomass productivity at 900 $\mu mol\, m^{-2}\, s^{-1}$. Nevertheless the best lipid productivity was reached at 1500 $\mu mol\, m^{-2}\, s^{-1}$, also by *Tetraselmis* sp., being 29.5 $mg\, L^{-1}\, day^{-1}$, where biomass productivity was of 124.5 $mg\, L^{-1}\, day^{-1}$. All three microalgae species were able to remove > 90% of nitrogen and orthophosphates, and 80% of carbon, which makes them suitable for treating mariculture wastewater, and in addition, represent a valuable high lipid content biomass byproduct usable as raw material for biodiesel synthesis.

1. Introduction

Aquaculture production has increased substantially for the past 40 years [1,2]. This industry is responsible of producing wastewaters with high contents of suspended solids and dissolved nutrients, which are discharged untreated into water bodies, causing eutrophication [3], generating damages to coastal ecosystems, and eventually the loss of biodiversity [4].

Conventional biological and physicochemical wastewater treatments can remove nutrients contained in this kind of wastewaters, and produce a good quality effluent. However, these methods involve the production of sludges which in turn must be treated prior to discharge; generating additional costs. In this regard, microalgae culture in aquaculture wastewaters offers the advantages of removing nutrients while simultaneously producing biomass that may be used as raw material for valuable products, such as biofuels [5].

The economic feasibility of microalgae biofuels faces various challenging limiting factors, such as the need for cultures with high lipid productivity in the shortest time period [6,7]. It is well-known that cell growth and lipid accumulation do not take place simultaneously during culture, so the techniques for increasing lipid accumulation often render low biomass quantities, and consequently, low lipid productivity [8]. To address this issue, the microalgae culture is carried out in two stages. In the first stage, microalgae are cultured in optimum growth conditions; and in the second stage, the cultures are subject to stress conditions in order to enhance the accumulation of energy in the form of neutral lipids (triacylglycerols) by altering their biosynthetic pathways. The result is no increase in microalgae density, but an important

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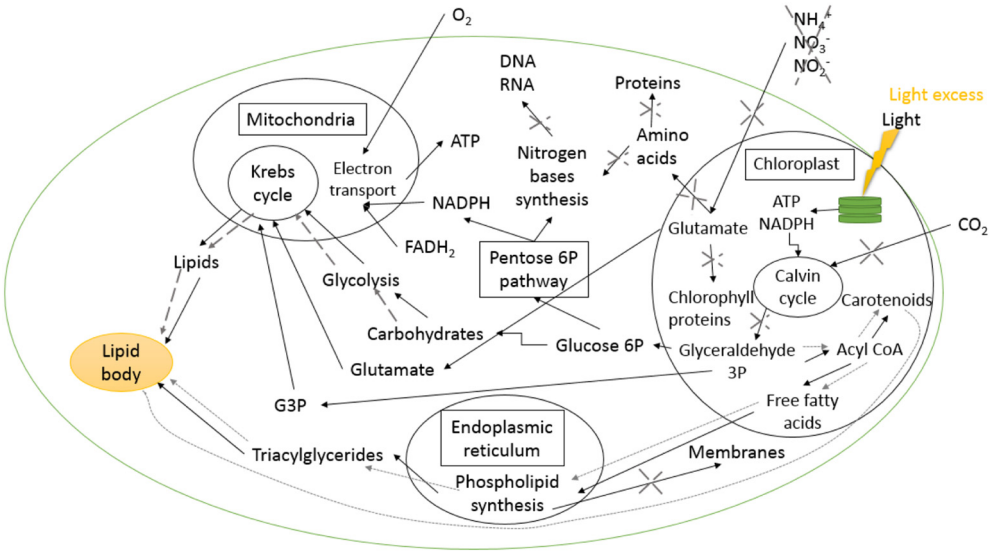

Fig. 1. Conceptual model of lipid synthesis during nitrogen limitation and light excess in a microalgae.

Table 1
Doubling times of the 3 microalgae species (Data shown is the mean, n = 3).

Microalgae	Photosynthetically active photon flux density (PAPFD) ($\mu mol\ m^{-2}\ s^{-1}$)		
	900	1500	2000
Tetraselmis sp.	3.21	3.56	4.23
Dunaliella sp.	4.13	4.13	4.80
Nannochloropsis sp.	5.83	5.27	6.67

Table 2
Specific growth rate and biomass productivity of the 3 microalgae species (Data shown is the mean, n = 3).

Microalgae	PAPFD ($\mu mol\ m^{-2}\ s^{-1}$)	900	1500	2000
Dunaliella sp.	Specific growth rate (day^{-1})	0.19	0.20	0.17
	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	68.9	69.3	52.1
Nannochloropsis sp.	Specific growth rate (day^{-1})	0.15	0.18	0.17
	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	88.0	99.5	84.9
Tetraselmis sp.	Specific growth rate (day^{-1})	0.29	0.28	0.23
	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	132.8	124.5	101.2

increase in lipid content [9,10].

There are several physicochemical factors that affect microalgae metabolism, for enhancing lipid production, such as light intensity, photoperiod, temperature, salinity, nutrient concentration in the growth medium (carbon, phosphates, nitrogen), and nitrogen (ammonium or nitrate) and carbon (glucose or glycerol, among others) sources. By exposing microalgae to environmental stress in laboratory scale cultures, through the modification of said factors, high lipid productivity has been achieved [11]. Nitrogen limitation causes three main changes: decrease in the thylakoid membrane; stimulation of phospholipid hydrolysis; and fatty acid synthesis enzyme activation. These changes cause an increase of the intracellular fatty acids content [12]. Generally, when microalgae are grown under low light intensities, the assimilated carbon is used for the synthesis of amino acids and other essential components, but under light saturation conditions sugars, lipids, and starch are formed [13]. An adequate light intensity contributes to lipid overproduction, which may be the result of the over-generation of photo-assimilated compounds that are converted into chemical energy [14].

Fig. 1 shows a conceptual model of lipid accumulation in microalgae. During the nitrogen limitation stage, the pathways marked with dashed arrows are activated for lipid production; other pathways are the membrane lipid recycling, and the protein degradation. Pathways indicated with X are inhibited mostly because there is a reduction in the photosynthetic rate, and carbon fixation through photosynthesis is reduced, as well as protein synthesis. When there is light excess the

Table 3
Nutrient removal kinetics and removal percentages of the 3 microalgae species (Data shown is the mean, n = 3).

PAPFD ($\mu mol\ m^{-2}\ s^{-1}$)		*Dunaliella* sp.		*Nannochloropsis* sp.		*Tetraselmis* sp.	
		Removal percentage (%)	Removal kinetics ($mg\ L^{-1}\ day^{-1}$)	Removal percentage (%)	Removal kinetics ($mg\ L^{-1}\ day^{-1}$)	Removal percentage (%)	Removal kinetics ($mg\ L^{-1}\ day^{-1}$)
900	Total nitrogen[a]	96.91	3.50	94.16	3.85	97.99	7.07
	Orthophosphates	94.02	0.87	97.12	0.78	95.62	1.49
	COD	81.85	17.35	81.45	10.45	81.56	20.11
1500	Total nitrogen[a]	97.26	3.57	90.07	3.68	98.03	7.25
	Orthophosphates	94.25	0.94	97.16	0.81	95.94	1.48
	COD	82.38	17.99	81.02	10.17	80.97	19.34
2000	Total nitrogen[a]	97.53	3.51	94.24	4.02	98.06	7.34
	Orthophosphates	93.27	0.89	97.20	0.83	95.67	1.48
	COD	81.29	16.71	81.85	10.74	75.60	14.08

[a] Total nitrogen: is the sum of the concentrations of nitrates, nitrites and ammonia.