Dairy wastewater as growth substrate for biomass and biocompound production by *Spirulina platensis*

Escolà G.[1], Zapata D.[2], Aristizábal A.[3], Arroyave C.[2], Poschenrieder C.[1], Llugany M.[1,*]

[1] Plant Physiology Laboratory, Facultat de Biociències, Universitat Autonòma de Barcelona, Campus de la UAB, Plaça Cívica, 08193 Bellaterra, Barcelona, Spain.
[2] Environmental Engineering Department, Engineering Faculty, Universidad de Medellín, Carrera 87 # 30-65, Medellín, Colombia.
[3] Process Engineering Department, Engineering Faculty, Universidad EAFIT, Carrera 49 # 7 Sur-50, Medellín, Colombia.

*corresponding author: e-mail: merce.llugany@uab.cat

Abstract
Spirulina former *Arthrospira* is a Cyanobacteria with economic applications in agriculture, biofuel production and wastewater treatment. In this study the influence of the carbon source in the medium and the medium's conductivity for *Spirulina platensis* UTEX growth over time was investigated. Three different culture media were evaluated: i) modified UTEX medium with inorganic carbon in the form of sodium bicarbonate and carbonate, ii) modified UTEX medium where inorganic carbon was replaced by lactic acid, and sea water enriched with lactic acid. Growth responses, morphological parameters (degree of spiralization), chlorophyll and phytohormone production were assessed. **Keywords:** Chlorophyll, bioproducts, phytohormones, microalgae, water treatment.

1. Introduction

Spirulina production is of main interest in food, cosmetic, biofuel, agriculture and pharmaceutical industries. This is due to its high content of valuable bioproducts like proteins, essential fatty acids, essential amino acids, minerals, vitamins, antioxidants, pigments, polysaccharides and phytohormones (Soni *et al*., 2017) (Wuang *et al*., 2016). Moreover, phytohormones from microalgae are of main interest as biostimulants for crops, but there are few reports in the literature (Romanenko *et al*., 2016) (Grunert *et al*., 2016). Achieving efficient and economically feasible microalgae production is a major concern. *Spirulina* is suited for large-scale cultivation due to its high photosynthetic efficiency, fast growth rate, great adaptability to the environment and to different substrates (Shi *et al*., 2016) including agroindustry wastewaters, like dairy wastewater (Chokshi *et al*., 2016) (Hena *et al*., 2018). Using wastewaters as culture medium for cultivation of microalgae is desired due to the low-cost and high availability in volume and its macro- and micro-nutrients content (Salama *et al*., 2017). Zhou *et al*. (2012) found that organic carbon-rich wastewaters have great potential as microalgal media. Cyanobacteria can grow in a mixotrophic way, so it is able to combine autotrophic photosynthesis and heterotrophic assimilation of organic compounds (Andrade & Costa, 2007).

This adaptability to different carbon sources makes *Spirulina* interesting for bioproduct generation from wastewaters and for wastewater treatment (Salama *et al*., 2017). The aim of this research was to evaluate the influence of different carbon sources on the performance of *Spirulina platensis*.

2. Materials and Methods

Spirulina platensis UTEX strain was grown in three culture media with different carbon sources: modified *Spirulina* UTEX medium (UTEX, Culture Collection of Algae, University Texas Austin, 2018) with sodium bicarbonate and sodium carbonate as inorganic carbon source (M_1), modified *Spirulina* UTEX medium where inorganic carbon was replaced by lactic acid (M_2), and sea water enriched with lactic acid (SW). Media M_2 and SW were formulated simulating dairy waste water properties Cultures samples were taken at days 2, 4, 6 and 8 of cultivation to determine dry biomass, concentrations of chlorophyll-α (by UV-vis) and phytohormones (analysed by LC–ESI(-)–MS/MS system following the method described by Llugany *et al.* with modifications) and microalgal degree of spiralization.

3. Results and Discussion

Table 1 reports the growth over time of *Spirulina platensis* in different media. It is observed that in SW medium, *Spirulina* shows a significantly higher growth rate followed by M_2 and M_1. The differences in growth rates decreased with exposure time, but the tendency was maintained until day 8, when SW showed biomass productions of 52% and 15% higher than M_2 and M_1, respectively. The organic carbon source (Lactic acid) achieved higher biomass than carbonate and bicarbonate source. Differences in growth in M_2 and SW were due to higher saline concentration in seawater, with a 5 times higher conductivity than SW.

Table 1. Effect of the carbon source in the average *S. platensis* UTEX dry biomass production over time.

Time (d)	Dry Biomass Concentration (g/L)		
	M_1	M_2	SW
2	0.0488	0.1895	0.7005
4	0.1253	0.4633	0.7933
6	0.2650	0.5585	0.9314
8	0.5350	0.9514	1.1240

Chlorophyll-α production decreases with a higher dry biomass production. The inorganic carbon source favored chlorophyll-α production in comparison to M_1 and M_2; while a higher medium conductivity (SW) was detrimental for chlorophyll-α production (See Figure 1). The medium composition did not significantly affect *S. platensis* spiralization degree under the studied conditions.

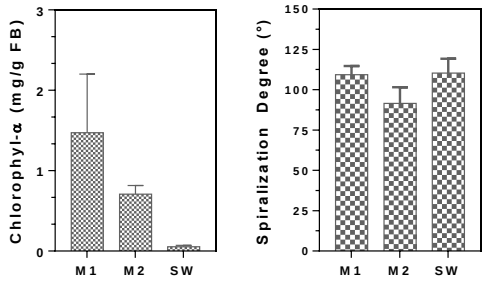

Figure 1. Effect of culture media on chlorophyll-α production and microalgal spiralization at day 8 of cultivation.

The phytohormone analysis revealed the presence of jasmonic (JA), indole acetic acid (IAA), phenylacetic (PAA) acids and benzylaminopurine (BA) in the *Spirulina* strain. Further research is required to identify and quantify other phytohormones potentially present in *S. platensis*.

4. Conclusions

Spirulina platensis biomass growth and chlorophyll-α production are strongly influenced by the type of carbon source and the conductivity of the medium but opposite trends are observed. As JA, IAA, PAA and BA phytohormones were detected in all conditions, this strain results highly interesting for crop applications as biostimulant.

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