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Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process

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HIGHLIGHTS

- Wastewater treatment with microalgae produced biomass to be used as biodiesel source.
- Wastewater cultured *S. obliquus* increased lipid content in response to stress factors.
- CO_2, light and salt factors acting isolated increased lipids.
- Salt presence in darkness increased lipids avoiding the use of photobioreactors.
- ω-3 eicosapentaenoic acid content of biomass slightly exceeded EU biodiesel normative.

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ABSTRACT

The microalga *Scenedesmus obliquus* was cultured in two cultivation stages: (1) in batch with real wastewater; (2) maintaining the stationary phase with different conditions of CO_2, light and salinity according to a factorial design in order to improve the lipid content. The presence of the three factors increased lipid content from 35.8% to 49% at the end of the second stage; CO_2 presence presented the highest direct effect increasing lipid content followed by light presence and salt presence. The ω-3 fatty acids content increased with CO_2 and light presence acting in isolation, nevertheless, when both factors acted together the interaction effect was negative. The ω-3 eicosapentaenoic acid content of the oil from *S. obliquus* slightly exceeded the 1% maximum to be used as biodiesel source (EU normative). Therefore, it is suggested the blend with other oils or the selective extraction of the ω-3 fatty acids from *S. obliquus* oil.

1. Introduction

Microalgae cultivation is currently been proposed as promising source of biofuels and high-value products as nutraceuticals (Borowitzka, 2013). However, microalgae cultivation in a commercial scale for biofuel production appears not to be economically feasible and sustainable (Markou and Nerantzis, 2013). On the other hand, nitrogen and phosphorus present in wastewater could present a cheap raw material for microalgae cultivation making the production of microalgae as by-product of the wastewater treatment as a niche opportunity (Park et al., 2011). In terms of environmental benefits, wastewater microalgal cultivation for subsequent biofuel production implies a reduction in carbon and water footprint that increases the sustainability of the biofuel from algae production (Clarens et al., 2010).

Microalgae cultivated under stress conditions have the ability to alter their biomass composition and accumulate lipids and carbohydrates which could be used for biofuel production, therefore their potential to be used as biofuel feedstock increases (Markou and Nerantzis, 2013). Under optimal conditions of growth, algae synthesize fatty acids to produce membrane polar lipids, as glycolipids and phospholipids, while under stress conditions many algae alter their lipid synthesis pathways and accumulate neutral lipids, mainly in the form of triacylglycerols. These latter lipids do not perform a structural role as the first ones but serve as a carbon and energy storage. These triacylglycerol lipids can be extracted and isolated from harvested microalgae and then converted to biodiesel by transesterification (Hu et al., 2008). The fatty acid profile of the microalgal oil plays a crucial role in the performance of biodiesel properties. These critic parameters that vary according to the carbon chain sizes and the disposition of double bonds are cetane number (*CN*), iodine value (*IV*) and cold filter plugging point (*CFPP*) (Knothe, 2005; Ramos et al., 2009).

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Nutrient starvation during stationary growth phase is well known as lipid enhancer, in this sense there are other factors as temperature, light, salinity and growth phase that have been shown to influence the algal metabolism (Boyle and Morgan, 2009). This lipid accumulation occurs at the expense of energy used for growth, leading to a decrease in growth rate and thus in biomass and lipid productivity (Wijffels and Barbosa, 2010). Cultivation in multiple-stage process is suggested to avoid the hamper of productivity reduction. Several authors propose a first stage for biomass growth in optimum conditions followed by the application of subsequent stress conditions promoting lipid accumulation (Prathima Devi et al., 2012; Markou and Nerantzis, 2013).

The species *Scenedesmus obliquus* has been commonly proposed as a candidate strain to treat wastewaters (Arbib et al., 2013; Ruiz et al., 2014). Moreover this species can accumulate quantities of lipids under stress conditions as nitrogen deficiency (Mandal and Mallick, 2009) and presents an adequate fatty acid profile in terms of linolenic acid and polyunsaturated acids to produce biodiesel (Gouveia and Oliveira, 2009).

Considering that the energetic requirements of microalgae production processes can determine the sustainability of the whole process, it becomes mandatory to apply the stress factors in a second cultivation stage with minimum energy consumption. Under our knowledge there are no previous works describing the influence of stress factors on microalgae biomass once the objective of the wastewater treatment is achieved. For this study there have been selected 3 stress factors to be applied, these are (1) the light presence, which is determined by the maintenance of the culture in the photobioreactors or by transferring the cultures to an opaque liquid container; (2) the salinity, which can be easily increased by the addition of available and cheap resources as NaCl or marine water; and finally (3) the use of CO_2, which is an already required resource in microalgae cultures.

In order to study the effect of these factors on lipid content and lipid profile of microalgae biomass, on a second cultivation stage subsequent to a wastewater treatment, a full factorial design based on the presence or absence of light, salinity and CO_2 has been applied to cultures of *S. obliquus* in the stationary phase after a batch growth in real wastewater. The design of the experiments included the effect of lipid enhancer factors as the nutrient starvation and the aging of the cultures.

2. Methods

2.1. Microorganism

S. obliquus (SAG 276.10) was obtained from Sammlung von Algenkulturen, pflanzenphysiologisches Institut, (Universität Göttingen, Germany). Stock cultures were maintained routinely in liquid nutritive COMBO medium by regular subculturing at 2-weeks intervals. Cultures were maintained at 20 ± 1 °C temperature and 143 μmol/m^2 s PAR light intensity under 14/10 light/dark cycle.

2.2. Wastewater

The feedstock used was secondarily pre-treated wastewater (WW) from the wastewater treatment plant "El Torno" located in Chiclana de la Frontera in southern Spain (municipality of around 80,000 inhabitants) (36°25′37.340″N, −6°9′23.386″W). The effluent was collected after the preliminary screening, primary sedimentation, activated sludge and secondary sedimentation processes. The WW was filtered by 1 μm nominal pore glass fiber filter previous to be used as culture medium, and characterized as follows: 20.09 ± 1.3 mg Total-N/L, 15.79 ± 0.07 mg NH_4-N/L, 2.17 ± 0.02 mg NO_2-N/L, 0.24 ± 0.01 mg NO_3-N/L, 1.89 mg N/L (organic nitrogen), 1.55 ± 0.01 mg Total-P/L, 10.67 ± 0.33 mg C/L (total organic carbon), 70 ± 2.1 mg O_2/L (chemical oxygen demand) and pH = 9.27. Dissolved species of N were determined by ionic chromatography (Metrohm, 881 Compact IC pro Anion and MCS 882 Compact IC plus Cation). Total N and P were determined as explained in the nutrient determination section and organic N was calculated by the subtraction of the N inorganic species from total concentration. Total organic carbon was determined by means of high temperature catalytic oxidation in an analyzer (Shimadzu TOC-5050A), chemical oxygen demand was determined with Spectroquant® COD test kits (Merck, 1.14541.0001) and pH was measured with a pH meter (Crison, GLP 21).

2.3. Experimental set-up

Experiments were conducted in batch by using 2000 mL borosilicate cylindrical flasks as photobioreactors (12.5 cm diameter and 14.5 cm height). Illumination was provided from the top of the flasks by using eight fluorescent lamps (four PHILIPS Master TLD 58 W/840 and four SYLVANIA Gro-Lux F 58 W/GRO-T8) with 143 μmol m^{-2} s^{-1} PAR light intensity and 14/10 light/dark cycle. PAR light intensity was measured by a digital light meter (Hansatech QRT1 Quantitherm light meter). 1.5 L of wastewater was inoculated with 90 mL suspension of pre-cultured cells, to obtain an initial biomass concentration in all reactors of around 0.1 g/L (biomass dry weight). The experiments were conducted at (20 ± 1 °C) in a thermostatic chamber. Aeration was supplied from the bottom of the flask at a flow rate of 1.5 L/min and enriched with CO_2 at 4%, this value is tolerated by this species (Tang et al., 2011) and simulates the flue gas of a natural gas combined heat and power plant (Shao et al., 1995).

2.4. Experimental design

To study the effects of salinity, CO_2 and light to already grown biomass, a replicated multilevel factorial design which included all the possible combinations among these three factors was applied (Table 1).

This factorial design was employed to screen factors that may have significant effects on response(s). Predefined values of the factors are expressed in terms of levels. The quality of the results from the factorial design was evaluated using a factorial analysis

Table 1
Experimental domain, (−) and (+) are codes of the factorial experimental design meaning absence and presence, respectively, of CO_2 (C), salinity (S) and light (L) factors.

Experiment	Codification	CO_2 (% in aeration)	Salinity (g/L marine salt)	Light
1	E1 (+C+L+S)	4	15	Presence
2	E2 (+C−L+S)	4	0	Presence
3	E3 (+C+L−S)	4	15	Absence
4	E4 (+C−L−S)	4	0	Absence
5	E5 (−C+L+S)	0	15	Presence
6	E6 (−C−L+S)	0	0	Presence
7	E7 (−C+L−S)	0	15	Absence
8	E8 (−C−L−S)	0	0	Absence