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Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production

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ABSTRACT

Microalgae are a promising bioenergy source as well as wastewater pollution reducers. This study aims to determine which species better satisfies the double objective of lipid production and wastewater nutrient removal. Seven species were cultured in batch under laboratory conditions in real wastewater and synthetic medium aiming to perform a meaningful comparison among them. Biomass productivity was higher in the wastewater than in the synthetic medium for the strains *Chlorella vulgaris*, *Chlorella kessleri* and *Scenedesmus obliquus*, the latter species yielding the highest biomass concentration (1.4 g/L), lipid content (36.75%) and lipid productivity (29.8 mg lipids/L·d) while *C. vulgaris* reached the highest biomass productivity (0.107 g/L·d). On the other hand, *Neochloris oleoabundans* did not grow in wastewater. Algae were not able to remove nitrogen from wastewater and as they were not nitrogen starved their lipid content was lower than when cultured in synthetic medium. The species that achieved maximum daily nitrogen removal from wastewater was *C. sorokiniana* (6.6 mg Total-N/L·d) followed by *S. obliquus* (4.4 mg Total-N/L·d). *S. obliquus* was better than *C. sorokiniana* at achieving the double objective of nutrient removal and lipid production in wastewater.

1. Introduction

Earth fossil fuel resources are gradually being exhausted while fossil fuel related emissions are continuously increasing [1]. Under this scope, utilization of wastewater containing nutrients and CO_2 emissions for microalgae production could diminish environmental degradation while raw materials for biofuel industry are generated.

Microalgae-based processes are considered as green technologies since they constitute a reuse of CO_2, instead of fossil raw materials [2]. Even if carbon capturing with microalgae and its further uses cannot reduce CO_2 emissions because the biomass produced does not offer long-term CO_2 storage, these strategies can contribute towards less dependence on fossil fuels and can contribute to the production of biofuels in a more sustainable way [3].

Certain studies point out that the use of wastewaters as source of nutrients is one of the most important ways to reduce life-cycle impacts and costs [4,5] offering synergetic advantages for algal biofuel feedstock production [6]. In this sense, there is reported a wide experience on microalgae cultivation in wastewater under laboratory conditions [7,8] and in higher scale [9]. In addition, CO_2 utilization for microalgae cultivation is demonstrated to increase biomass productivity [10,11].

In microalgae cultivation processes, nitrogen (N) and phosphorus (P) are required in higher amounts than for typical terrestrial crops [12]. On the one hand, supply of N fertilizer is energy-intensive because of its manufacture process [13]. On the other hand, it must also be considered that P fertilizer is not renewable and a future global limitation of P is expected due to its mineral origin [14]. Under this scope, the need to use different nutrient sources derived from wastes must be a priority issue in order to reduce resource competition against agriculture and to efficiently exploit non-renewable resources.

Microalgae growth rate and oil content are important parameters to take into account in species bioprospection for algae-based industry. Biomass yields may be considered as an adequate criterion for biodiesel production if they are associated with lipid productivity [15]. Several reports describe the growth of microalgae under the scope of an industrial production but comparisons between them cannot be properly made due to different experimental conditions and calculation methodologies.

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Therefore, the aim of this work was to carry out a selection between seven microalgae species under the same experimental conditions, evaluating the aptitude to grow in wastewater in terms of biomass production, nutrient uptake and the capability to produce lipids in this medium.

2. Methods

2.1. Microalgae

Chlorella vulgaris, (SAG 211-12), *Botryococcus braunii* (SAG 30.81), *Chlorella kessleri* (SAG 211-11g), *Chlorella sorokiniana* (SAG 211.8k), *Scenedesmus obliquus* (SAG 276.10), *Ankistrodesmus falcatus* (SAG-202-2) were obtained from Sammlung von Algenkulturen, pflanzenphysiologisches Institut, (Universität Göttingen, Germany) and *Neochloris oleoabundans* (strain 1185) was obtained from the culture collection of the University of Austin, Texas.

Stock cultures were maintained routinely on both liquid and agar slants of COMBO medium [16] by regular subculturing at 2-week intervals. Cultures were maintained at 20 ± 1 °C temperature with 143 μmol·m^{-2}·s^{-1} PAR light intensity under 16:8 light dark cycle.

2.2. Culture media

2.2.1. Wastewater

The feedstock used was secondarily treated urban wastewater (WW) from the wastewater treatment plant "El Torno" located in Chiclana de la Frontera in Cádiz (municipality of around 80,000 inhabitants) (36° 25′ 37.340″ N, −6° 9′ 23.386″ W), Spain. The WW was collected from the effluent after the preliminary screening, primary sedimentation, activated sludge and secondary sedimentation processes. WW was filtered by 1 μm nominal pore glass fiber filter previous to be used as culture medium, and characterized (Table 1), this step would probably have removed all microalgae predators from WW, therefore microalgae ability to compete in WW as ecological systems is not taken into account for this study. 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. Organic N was calculated by the subtraction of the N species from total concentration. Total organic carbon was determined in a TOC-analyzer (Shimadzu TOC-5050A) and chemical oxygen demand was determined with Spectroquant® COD test kits (Merck, 1.14541.0001).

2.2.2. Synthetic medium

COMBO medium was selected as synthetic medium (SM) for comparison with WW. This medium was devised by Kilham et al. [16] and is a widely used synthetic media that supports robust growth of freshwater algae. COMBO medium was composed of (per liter): 85.01 mg $NaNO_3$, 8.71 mg K_2HPO_4, 36.76 mg $CaCl·2H_2O$, 36.97 mg $MgSO_4·7H_2O$, 12.6 mg $NaHCO_3$, 24 mg H_3BO_3, 7.45 mg KCL, 4.36 mg $Na_2EDTA·2H_2O$, 1 mg $FeCl_3·6H_2O$, 1 mg $CuSO_4·5H_2O$, 22 mg $ZnSO_4·7H_2O$, 12 mg $CoCl_2·6H_2O$, 180 mg $MnCl_2·4H_2O$, 22 mg $Na_2MoO_4·2H_2O$, 1.6 mg H_2SeO_3, 1.8 mg $NaVO_4$, 100 μg Thiamin·HCl (B_1), 0.5 mg Biotine (Vitamin H) and 0.55 mg Cyanocobalamin (B_{12}).

Final measured concentration of total N and total P in COMBO medium were 13.25 ± 1.25 and 1.41 ± 0.19 mg/L ($n = 3$), respectively.

Table 1
Characterization of the WW medium. Data are expressed as mean and standard deviation (n = 3) when possible.

Parameter	Units	Concentration
Total nitrogen	(mg N/L)	20.09 ± 1.3
Ammonium	(mg NH_4-N/L)	15.79 ± 0.07
Nitrate	(mg NO_2-N/L)	2.17 ± 0.02
Nitrite	(mg NO_3-N/L)	0.24 ± 0.01
Organic nitrogen	(mg N/L)	1.89
Total phosphorus	(mg P/L)	1.55 ± 0.01
TOC	(mg C/L)	10.67
COD	(mg O_2/L)	70

2.3. Experimental set-up

The experiments were conducted in batch by using 2000 mL borosilicate flasks (12.5 cm diameter × 14.5 cm height). At the beginning of each series of experiments, 1500 mL of culture medium 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. Illumination was provided from the top of the flasks by using eight fluorescent lamps with 143 μmol·m^{-2}·s^{-1} PAR light intensity and 14/10 light/dark cycle. Light intensity was measured by a digital light meter (Hansatech QRT1 Quantitherm light meter). The experiments were conducted at 20 ± 1 °C in a thermostatic chamber. Aeration was supplied, from the bottom of the flask, by an air compressor at a flow rate of 1.5 L/min. The air was enriched with CO_2 at a similar concentration of the flue gas from a natural gas combined heat and power plant, 4% [17].

2.4. Biomass

Biomass concentration was daily evaluated and measured through the correlation between the optical density (OD) and the dry weight of algal biomass suspended solids (SS) for each species and culture medium. Standard curves were defined for each species and culture medium and all linear regressions presented R^2 values higher than 0.98. OD was measured at 680 nm by means of a spectrophotometer (Thermo GENESYS 10-Vis) and algal biomass dry weight was determined gravimetrically as SS according to the standardized method 2540-D [18].

2.5. N and P determinations

Liquid samples for nutrient consumption analysis were withdrawn from each reactor and filtered by 0.45 μm to remove suspended solids. Total N and total P were determined colorimetrically after oxidation and analyzed as nitrate and phosphate based on the method proposed by [19]. 10 mL of the sample were mixed with 1.5 microspoon of Oxisolv® (Merck KGaA, Darmstadt, Germany), then incubated at 100 °C for 60 min and cooled to room temperature. Once samples were completely oxidized, nitrate and phosphate determinations were performed according to Spectroquant® test kits (Merck, 1.14773.0001 and 1.14848.0001, respectively).

2.6. Total Lipid determination

At the end of the experiments, biomass was harvested by centrifugation at 4000 rpm for 10 min, washed with deionized water twice and dried in a lyophilizer (Labconco, FreeZone Triad Cascade Benchtop) under high vacuum ($2·10^{-3}$ mbar; −40 °C) during 72 h. Total lipids (TL) were extracted from the lyophilized biomass according to the modified method reported by Takagi et al. [20]. 90 mg of lyophilized biomass pellet was mixed with 12 mL of 2:1 trichloromethane/methanol and 0.6 g of analytical grade quartz (particle size 10–30 μm) were added [21]. Samples were sonicated for 90 min in an ultrasound bath (35 kHz; 80 W). The solvent was collected and the extraction was repeated twice. Final extracts were centrifuged at 5000 rpm for 10 min and filtered through 1 μm glass fiber filter for solids separation. Then, solvents of the final organic phase were removed under vacuum in a rotary evaporator at 65 °C. The remainder was dried in a desiccator for 24 h and weighed as TL. All extractions were done in duplicate.

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