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Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: A kinetic study

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ABSTRACT

Microalgae are considered as one of the most promising sources of biomass for energy production. However, bioenergy production by microalgal culture is still not economically viable and it has high environmental impact (requirement of high amount of freshwater). These drawbacks can be surpassed by coupling microalgal biomass production with phycoremediation of wastewater. In this context, this study evaluates the kinetics of biomass production and nutrient removal by two microalgal species (*Chlorella vulgaris* and *Pseudokirchneriella subcapitata*) cultivated in different medium compositions.

The potential of microalgae for biomass production and their high efficiency on nutrients removal from medium, particularly nitrogen and phosphorus, was demonstrated. Maximum biomass productivity was observed for *C. vulgaris* (0.106 ± 0.004 g L^{-1} d^{-1}), while *P. subcapitata* reached a maximum of 0.050 ± 0.001 g L^{-1} d^{-1}. The value of N:P molar ratio that favoured microalgal growth was 8:1 for *C. vulgaris* and 16:1 for *P. subcapitata*. A complete removal (100%) of ammonium was measured and high removal efficiencies were observed for nitrate (above 95%) and phosphate (above 97%). Microalgae were also able to efficiently remove sulphates, presenting removal efficiencies from 54 to 100%. The removal kinetics for all the nutrients have been determined through application of pseudo-first-order kinetic model and modified Gompertz model. In conclusion, this work gives relevant data for culturing microalgae in wastewater, contributing to the bioprocess design of a sustainable and low-cost production of microalgal biomass.

1. Introduction

Alternative sources of energy with lower carbon intensity and thus, more sustainable, should be studied. Biomass is a renewable energy resource that, with adequate management, can achieve high regeneration rates being considered sustainable (zero-emission energy source) [1–3]. In this context, microalgae appear as an important source of biomass. These photosynthetic microorganisms present higher growth rates and higher biomass productivities when compared to terrestrial crops [4–8]. Microalgae can be grown in non-arable land and require far less land than terrestrial crops, thus not competing with agriculture and not compromising food production and supply. Additionally, microalgae can grow in a wide variety of environmental conditions and also in low quality waters, reducing the requirements for freshwater [9,10]. Due to their macromolecular composition, several commercial products can be achieved from microalgal biomass [11]: human food, animal feed, fine chemicals, biofuels and fertilizers. Microalgal cultures are already performed at large-scale, mainly for high-valued human nutritional products. However, bioenergy production is not economically viable yet; thus, several research efforts should be performed to reduce biomass production costs. Besides the search for the culture parameters corresponding to maximum growth rates, the process integration of biomass production with wastewater treatment (secondary or tertiary treatment) will provide a significant reduction on the requirement for freshwater and nutrients (whose price almost doubled in the last decade) [12,13]. On the other hand, wastewater treatment using microalgae has several advantages over conventional treatments [14–16]: (i) nitrogen and phosphorus can be converted into biomass without the addition of organic carbon; (ii) the discharged effluent into water bodies is oxygenated; and (iii) high-valued products can be extracted from microalgal biomass. The main mechanisms for nutrient removal from microalgae include uptake into the cell and, in the case of ammonia, the stripping through elevated pH [17,18]. However, tertiary treatment of wastewater with microalgae should guarantee that the discharge limits for urban wastewaters defined by the European Union (EU) Directives 91/271/EEC and 1998/15/EC are accomplished. Taking into account the definition of population equivalent (p.e.) presented in the EU legislation, the limits for effluent discharge are: (i) 2 mg_P L^{-1} (for 10 to 100 thousand p.e.) or 1 mg_P L^{-1} (for more

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than 100 thousand p.e.) for total phosphorus and a removal efficiency of this nutrient in the overall load of at least 80%; and (ii) 15 $mg_N\ L^{-1}$ (for 10 to 100 thousand p.e.) or 10 $mg_N\ L^{-1}$ (for more than 100 thousand PE) for total nitrogen and a removal efficiency of this nutrient in the overall load of at least 70–80%. One or both parameters (values for concentrations or the percentage of reduction) may be applied depending on the local situation.

According to their source, wastewaters can present different compositions, some of them with compounds that inhibit microalgal growth. Several research studies were already performed with microalgal growth in wastewaters from different sources: (i) domestic wastewater [19–21]; (ii) anaerobic digestion wastewater [22–24]; (iii) livestock wastewater [25–27]; and (iv) agro-industrial wastewater [28,29]. In almost all studies, microalgae were able to efficiently remove the monitored nutrients. Lundquist et al. [30] performed a techno-economic assessment of biofuel production by microalgae using wastewater as culture medium, selecting five case studies: two of them focused on wastewater treatment and the others on biofuel (biogas and biodiesel) production. Without integration with wastewater treatment, microalgal biofuels can exceed $400 per barrel, while this integration can lower the price to less than $30 per barrel. Thus, an important step to increase the competitiveness (promoting simultaneously the environmental sustainability) of microalgal biofuels over fossil fuels is the optimization of culture parameters using wastewater as culture medium.

Several phenomena should be studied to apply this technology at industrial scale. Kinetics of microalgal growth and nutrient removal are required to perform the bioprocess design. In addition, the influence of nitrogen to phosphorus (N:P) molar ratio on the growth of microalgae and the effect of fed nitrogen source (nitrate or ammonium) should be analysed. Therefore, this study aimed to evaluate the kinetics of biomass production and nutrient removal of microalgae grown under different experimental conditions. Specific objectives were: (i) to evaluate the effect of nitrogen to phosphorus (N:P) molar ratio and nitrogen source on the growth of two microalgae (*Chlorella vulgaris* and *Pseudokirchneriella subcapitata*); and (ii) to evaluate the kinetic parameters for biomass production and nutrient uptake from the culture medium.

2. Materials and methods

2.1. Microorganisms and culture medium

C. vulgaris and *P. subcapitata* were obtained from the Culture Collection of Algae and Protozoa (CCAP). The selection of these microorganisms was based on the following factors: (i) both microorganisms can be easily grown in laboratory cultures; (ii) different studies have shown that microorganisms from the genus *Chlorella* have been effectively applied in nutrients removal from wastewaters from different sources [31–33]; and (iii) *P. subcapitata* is a green microalga commonly used as a chemical toxicity bioassay organism [34,35] that has shown to be adapted to grow under different nitrogen and phosphorus concentrations [36]. Microalgae were inoculated in a modified standard medium [37] with the following composition (mg L^{-1}): 12 $MgCl_2 \cdot 6H_2O$; 18 $CaCl_2 \cdot 2H_2O$; 15 $MgSO_4 \cdot 7H_2O$; 20 KH_2PO_4; 0.08 $FeCl_3 \cdot 6H_2O$; 0.1 $Na_2EDTA \cdot 2H_2O$; 0.185 H_3BO_3; 0.415 $MnCl_2 \cdot 4H_2O$; 0.003 $ZnCl_2$; 0.0015 $CoCl_2 \cdot 6H_2O$; 10^{-5} $CuCl_2 \cdot 2H_2O$; 0.007 $Na_2MoO_4 \cdot 2H_2O$ and 1300 $NaHCO_3$. Different medium compositions regarding nitrogen (see Table 1) were applied to mimic the compositions of real effluents, which present a wide variability. NH_4Cl and $NaNO_3$ solutions were added at different molar ratios, to evaluate which nitrogen source (NH_4^+ and NO_3^-) results in an increased biomass productivity. Due to the variable composition of wastewaters, the use of a synthetic medium was considered more appropriate to reproduce the experiments at lab scale and to obtain mathematical models. N:P molar ratio is an important parameter in microalgal growth. Redfield ratio (16:1) was considered as middle value. Two additional ratios were selected, one higher (24:1) and one lower (8:1), to cover a wide range of values found in different wastewaters [38]: (i) poultry; (ii) swine; (iii) tannery and others. In addition, the selected concentrations of nitrogen and phosphorus are in the same order of magnitude of the values found in the same wastewaters [38].

Table 1
Concentrations of NH_4Cl and $NaNO_3$ for the different assays.

Assay	Microalgae	Nitrogen source	Mass concentration (mg L^{-1})			NH_4^+:NO_3^- molar ratio
			C1	C2	C3	
1		NH_4Cl	63	126	189	2:0
2	*C. vulgaris*	$NH_4Cl/NaNO_3$	31.5/50	63/100	94.5/150	1:1
3		$NaNO_3$	100	200	300	0:2
4		NH_4Cl	63	126	189	2:0
5	*P. subcapitata*	$NH_4Cl/NaNO_3$	31.5/50	63/100	94.5/150	1:1
6		$NaNO_3$	100	200	300	0:2

Mass concentrations C1, C2 and C3 corresponded to N:P molar ratio of 8:1, 16:1 and 24:1, respectively.

2.2. Experimental setup and culture conditions

Microalgae were inoculated in 1-L borosilicate glass flasks with an initial biomass concentration of approximately 20–30 mg L^{-1}. Cultures were performed at room temperature for 12 days using the above described medium. Agitation of the cultures was obtained by injection of atmospheric air at the base of the flasks, using air pumps Trixie TARP D-2463 (50–300 L) with an air flow of 90 L h^{-1}. Cultures were exposed to continuous light supply (provided by a set of four 18-W fluorescent lamps) with light intensity at the surface of the flasks between 2.5 and 3.0 klx. Light intensity was daily monitored using a light meter Isotech Lux-1335 — RS Components. The assays were performed in duplicates.

2.3. Analytical methods

The cultures were subjected to daily measurements of temperature, dissolved oxygen concentration (sensor Oxi 340i — WTW), pH (sensor pH 212 — Hanna Instruments) and optical density at 750 nm (OD_{750}). OD_{750} was measured using a spectrophotometer (Genesys 10S UV–Vis Scanning — Thermo Scientific). Biomass concentration was then calculated using the determined calibration curves for each microalga. The relationship between biomass dry weight ($g_{biomass}\ L^{-1}$, x) and optical density (OD_{750}, y) was estimated using the following linear regressions: $y = (1.80 \pm 0.08)x + (0.04 \pm 0.07)$ ($R^2 = 0.998$; limits of quantification and detection were 0.15 and 0.04 g L^{-1}, respectively) for *C. vulgaris* and $y = (2.6 \pm 0.2)x + (0.1 \pm 0.1)$ ($R^2 = 0.995$; limits of quantification and detection were 0.16 and 0.05 g L^{-1}, respectively) for *P. subcapitata*.

To evaluate the temporal variation of the medium chemical composition, five samples were collected in different days. These samples were centrifuged for 15 min at 4000 rpm using a centrifuge by Hitachi Himac CT6E Koki Co., LMT and filtered through syringe filters of nylon membrane with a pore size of 0.45 μm (Acrodisc ®, Pall). The filtered solution was then analysed taking into account the following compounds: (i) sulphate, chloride, nitrate, phosphate and nitrite measured by ion chromatography using a Dionex ICS-2100 apparatus equipped with an IonPac® AS11-HC (4 × 250 mm) column at 30 °C and an anion self-regenerating suppressor (ASRS® 300, 4 mm) under isocratic elution of 30 mM NaOH at a flow rate of 1.5 mL min^{-1}; (ii) sodium, potassium, ammonium, magnesium and calcium measured by ion chromatography using a Dionex DX-120 device equipped with an IonPac® CS12A (4 × 250 mm) column at room temperature and a cation self-regenerating (CSRS® Ultra II, 4 mm) suppressor under isocratic elution of 20 mM methanesulfonic acid at a flow rate of 1.0 mL min^{-1}; and (iii) dissolved organic carbon (DOC) concentration determined by combustion catalytic oxidation at 680 °C and non-dispersive infrared (NDIR) methods in a TOC-V_{CSN} analyser equipped with an ASI-V autosampler (Shimadzu). Total dissolved carbon (TDC) and dissolved inorganic