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Biotechnological potential of *Synechocystis salina* co-cultures with selected microalgae and cyanobacteria: Nutrients removal, biomass and lipid production

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HIGHLIGHTS

- Single and mixed cultures of microalgae and/or cyanobacteria were studied.
- Biomass productivities of the studied consortia increased up to 51%.
- Nitrogen and phosphorus were effectively removed using the studied consortia.
- An increased production of fatty acids was observed in the consortia.

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ABSTRACT

Cultivation of microalgae and cyanobacteria has been the focus of several research studies worldwide, due to the huge biotechnological potential of these photosynthetic microorganisms. However, production of these microorganisms is still not economically viable. One possible alternative to improve the economic feasibility of the process is the use of consortia between microalgae and/or cyanobacteria. In this study, *Chlorella vulgaris*, *Pseudokirchneriella subcapitata* and *Microcystis aeruginosa* were co-cultivated with *Synechocystis salina* to evaluate how dual-species cultures can influence biomass and lipid production and nutrients removal. Results have shown that the three studied consortia achieved higher biomass productivities than the individual cultures. Additionally, nitrogen and phosphorus consumption rates by the consortia provided final concentrations below the values established by European Union legislation for these nutrients. In the case of lipid productivities, higher values were determined when *S. salina* was co-cultivated with *P. subcapitata* and *M. aeruginosa*.

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1. Introduction

Microalgae and cyanobacteria are photosynthetic microorganisms that can be used in a wide variety of applications. These microorganisms can be effectively used in CO_2 capture from the atmosphere or from flue gas emissions and can remove nutrients from wastewaters, playing an important role in wastewater treatment processes (Razzak et al., 2013). Nitrogen removal efficiencies between 80 and 90% and phosphorus removal efficiencies close to 100% have already been reported (Zhu et al., 2013). Due to the high lipid productivities associated to microalgae and cyanobacteria, with theoretical value of 50,000 L ha^{-1} yr^{-1}, these microorganisms appear as an important raw material for biofuels production (Mata et al., 2010). The high energetic value of microalgal/cyanobacterial biomass makes it suitable for application in human food and animal feed. Finally, microalgae and cyanobacteria produce different compounds, such as pigments, antioxidants, β-carotenes, proteins and vitamins, which can be used to produce high-value products (Pulz and Gross, 2004).

In addition to the wide variety of biotechnological applications described for microalgae and cyanobacteria, cultivation of these photosynthetic microorganisms has several advantages. They present higher growth rates and higher biomass productivities than other photosynthetic organisms, such as terrestrial crops (Mata et al., 2010). Additionally, they present higher lipid contents and nutritional values (Pulz and Gross, 2004). Microalgae and cyanobacteria 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. These microorganisms can also grow in a wide variety of environmental conditions and also in low quality waters, reducing the requirements

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for freshwater (Chisti, 2007). However, microalgal/cyanobacterial production still faces some problems related to the economic feasibility of the process and to the low biomass productivities achieved. Recently, several authors have reported the use of polycultures of microalgae and/or cyanobacteria to improve biomass and lipid productivities and nutrients removal from the culture medium. However, these studies refer to the use of polycultures for single applications. Johnson and Admassu (2013) have studied biomass and lipid productivities in single and mixed cultures of *Chlorococcum* sp., *Scenedesmus* sp., *Chlorella* sp. and *Phaeodactylum tricornutum*, concluding that mixed cultures were more productive than single ones in terms of biomass and lipids. Additionally, the study of a microalgal consortium composed by the freshwater microalgae *Chlorella* sp. and *Scenedesmus* sp. resulted in more effective removal of nitrogen and phosphorus from the culture medium when compared to single cultures of these microorganisms: after three weeks of culturing, nitrogen and phosphorus removal efficiencies achieved were 88.6–96.4% and 99.7–99.9%, respectively (Koreivienė et al., 2014). The use of polycultures combining microorganisms presenting different metabolic activities and adapted to different environmental conditions results in the development of a robust system that can operate under different environmental conditions and different nutrient supplies (Boonma et al., 2014; Fouilland, 2012; Johnson and Admassu, 2013). Therefore, important characteristics of these cultures include: (i) high tolerance to environmental fluctuations and to multiple nutrient sources; and (ii) resistance to invasion by other species. Due to the wide variety of photosynthetic microorganisms and possible combinations, the study of polycultures is still in an early stage. Additionally, it is very difficult to select the microorganisms integrating the consortia. One possible alternative is to combine, for example, photoautotrophs and mixotrophs, ammonia and nitrate users, or marine and freshwater, aiming to improve both biomass productivities and the resilience of the consortium (Fouilland, 2012). In this study, the potential of three different consortia in biomass and lipids production and nutrients removal from the culture medium was evaluated, trying to achieve an integrated system with improved metabolic performance. The consortia were defined by selecting a marine cyanobacterium, *Synechocystis salina*, which was co-cultivated with three freshwater microorganisms (*Chlorella vulgaris*, *Pseudokirchneriella subcapitata* and *Microcystis aeruginosa*). Selection of a marine microorganism was based on the following factors (Fouilland, 2012): (i) marine microalgae or cyanobacteria are more resilient to salinity changes and can be cultured in freshwater; and (ii) the high productivities observed in marine coastal waters, even when submitted to considerable salinity and nutrient oscillations, suggest that these microorganisms may be effectively used for biomass production using wastewaters as culture medium. Additionally, several authors have reported the use of *S. salina*, *C. vulgaris*, *P. subcapitata*, and *M. aeruginosa* in a wide variety of biotechnological applications, such as CO_2 capture, wastewater treatment, biofuels production and synthesis of bioactive compounds (Chinnasamy et al., 2010; McLarnon-Riches et al., 1998; Philippis and Vincenzini, 1998; Wahlen et al., 2011). As far as it is known, this is the first study reporting the potential of polycultures of the selected microorganisms in an integrated process combining biomass and lipids production and nitrogen and phosphorus removal from the culture medium.

2. Methods

2.1. Microorganisms and culture medium

The microalgae *C. vulgaris* CCAP 211/11B and *P. subcapitata* CCAP 278/4 were obtained from Culture Collection of Algae and Protozoa (United Kingdom), while the cyanobacteria *S. salina* LEGE 06079 and *M. aeruginosa* LEGE 91344 were obtained from the Laboratory of Ecotoxicology, Genomic and Evolution – CIIMAR (Centre of Marine and Environmental Research of the University of Porto, Portugal). Stock solutions of these microorganisms were prepared in OECD (Organisation for Economic Co-operation and Development) test medium (OECD, 2011), with the following composition (per litre): 250 mg $NaNO_3$, 12 mg $MgCl_2{\cdot}6H_2O$, 18 mg $CaCl_2{\cdot}2H_2O$, 15 mg $MgSO_4{\cdot}7H_2O$, 45 mg KH_2PO_4, 0.08 mg $FeCl_3{\cdot}6H_2O$, 0.1 mg $Na_2EDTA{\cdot}2H_2O$, 0.185 mg H_3BO_3, 0.415 mg $MnCl_2{\cdot}4H_2O$, 3 μg $ZnCl_2$, 1.5 μg $CoCl_2{\cdot}6H_2O$, 0.01 μg $CuCl_2{\cdot}2H_2O$, 7 μg $Na_2MoO_4{\cdot}2H_2O$ and 50 mg $NaHCO_3$. Culture medium was sterilized by autoclaving at 121 °C for 15 min. The cells were incubated in 500-mL flasks (VWR, Portugal) at room temperature (24.0 ± 1.0 °C), under continuous fluorescent light with an irradiance of 120 μE m^{-2} s^{-1} at the surface of the flasks. Agitation was obtained by bubbling atmospheric air (filtered through 0.22-μm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks.

2.2. Microalgal culturing in single and dual-species cultures

Previously described stock solutions were used as inocula for the different studied cultures. Single cultures, as well as co-cultures containing *S. salina* (*S. salina* + *C. vulgaris* – SC, *S. salina* + *P. subcapitata* – SP and *S. salina* + *M. aeruginosa* – SM), were studied. Batch experiments were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL. Cells were cultivated for seven days with an initial concentration of approximately 1.0×10^6 cells mL^{-1} (in co-cultures, initial cell concentration was approximately 2.0×10^6 cells mL^{-1}). Temperature, light and aeration conditions were the same as for stock solutions preparation. Two independent experiments were performed in duplicates under aseptic conditions.

2.3. Growth monitoring and kinetic growth parameters

Duplicate samples were collected at 24-h intervals and cellular concentration was determined using a Neubauer counting chamber (Marienfeld, Germany), under a Leica DM LB (Leica Microsystems, Germany) microscope. Specific growth rates, μ (d^{-1}), were determined using cell concentration values, according to Eq. (1) (Gonçalves et al., 2014):

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \tag{1}$$

where N_2 and N_1 correspond to cell concentration (in cells mL^{-1}) at times t_2 and t_1 (in days), the end and beginning of the exponential growth phase, respectively.

Samples were also collected in the first and last day of culturing to determine cell dry weight (dw) and calculate average biomass productivities, P ($g_{dw}\,L^{-1}\,d^{-1}$), within the cultivation time, as represented in Eq. (2) (Gonçalves et al., 2014):

$$P = \frac{X_f - X_i}{t_f - t_i} \tag{2}$$

where X_f and X_i correspond to biomass concentration (in $g_{dw}\,L^{-1}$) at times t_f and t_i (in days), the end and beginning of cultivation time, respectively.

2.4. Nutrients removal

The removal of nutrients was determined by quantification of nitrogen and phosphorus in the culture medium within the cultivation time. For each analytical assay, one-millilitre samples from each suspension were collected at 24-h intervals. Samples

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