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Development of marine multi-algae cultures for biodiesel production

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ABSTRACT

The concept of community ecology has become a central issue for sustainable biofuel production. However the development of appropriate multi-algae cultures for the industry remains challenging. Natural marine algae blooms collected from coastal areas of Cyprus were induced to form multi-algae cultures, and five (5) of those were developed and tested. Algae growth characteristics, biomass, and lipid productivity were assessed, and the dominant microalga was isolated in all cultures. Growth characteristics, lipid productivity, and FAME composition varied considerably among the different multi-algae cultures as well as their corresponding dominant species. Our results suggest that competitiveness and species complementarity could be crucial factors for biomass and lipid productivity. In material collected from the marina in Larnaca of Cyprus, the induced BL1_LCA bloom was the most promising culture, dominated by *Nannochloropsis* sp. and accompanied by cyanobacteria assemblages, exhibiting the highest biomass and lipid productivity compared to the other developed blooms. Biomass and lipid productivity of an axenic monoculture of *Nannochloropsis* sp. were 2.1 and 2.2 times lower compared to those measured in BL1_LCA. The growth parameters of BL1_LCA and its corresponding dominant species were further tested under culture media of different seawater and wastewater ratios. An increase of wastewater ratio in the culture media resulted in a significant reduction of lipid, FAME concentration as well as biomass productivity both in BL1_LCA and its dominant isolate. Overall, our findings suggest that the complex interactions within microalgae community might be crucial for biodiesel production; moreover, the general assumption that wastewater can be applied as an alternative nutrient source should be used cautiously since species-specific responses seem to take place.

1. Introduction

Large-scale cultivation of microalgae is an energy-demanding process, which requires large amount of water resources as well as nutrients. A critical step for the development of a cost-effective biodiesel production is the isolation of appropriate strains of high growth rates that are capable of accumulating significant amounts of neutral lipids. Several studies showed that most of the selected strains that were tested under laboratory conditions do not exhibit high biomass productivity and do not accumulate high lipid concentrations when transferred under field conditions [1–4]. This is due to contamination, inadequate temperature control and light saturation and or limitation as well as water evaporation in open pond systems, and oxygen build-up in photobioreactors [1]. These findings suggest that the use of uni-algal cultures as feedstocks for biodiesel production is difficult and unprofitable [5]. Thus, to increase the potential of using microalgae as biofuel feedstocks it is necessary to develop simple and efficient ways to achieve proper cultivation systems with high lipid productivity.

It has been suggested that the implementation of ecological principles during microalgae biomass production could lead to a more stable system in terms of productivity [6–8]. The proposed use of "multispecies communities" with carefully chosen co-habitants was expected to increase biomass productivity. Indeed, earlier studies showed that multi-algae communities generally exhibited higher productivity [9], and that the ecological advantages of microalgae consortia incorporated into cultivation systems might have the potential to improve biodiesel productivity [6,10]. However, very few studies further explored this possibility of developing and applying multi-algae cultures in biodiesel processes [11,12]. A primary concern during the development multi-algae systems is to include species that are functionally different and exhibit a differential response to environmental changes leading to enhanced ecosystem stability [7]. This could be

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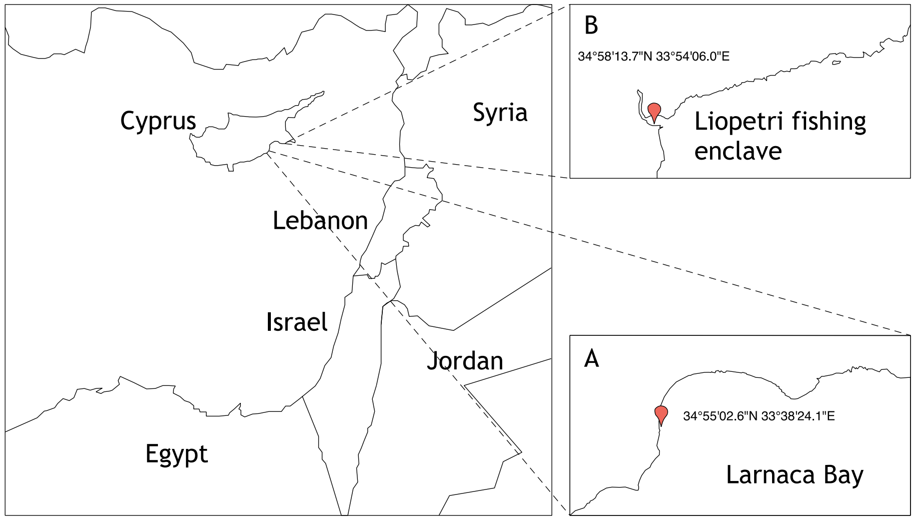

Fig. 1. Coordinates of the two coastal sampling locations in Cyprus: the marina of Larnaca (A) and the fishing enclave of Liopetri (B).

particularly important when novel technologies are implemented for biofuel production. Recent studies demonstrated that hydrothermal liquefaction is an alternative process, providing a high biocrude yield that could be further upgraded to produce appropriate for the market, hydrocarbon fuels [13,14]. Indeed, the process can be applied to all types of algae without the restriction to high-lipid producing strains [15]. Recently, it has been shown that strain specific characteristics like lipid content and higher biomass positively affects biocrude yields [16]. Thus the development of high biomass yielding multi-algae cultures in the long term could be a valuable source of biomass for biofuel production [17].

Besides the search for the most appropriate strain or species, the use of wastewater might be a viable resource to enhance the environmental and economic sustainability of the microalgae-derived biodiesel [18]. Indeed, many microalgae species are capable of growing in wastewaters utilizing the available abundant organic carbon and other nutrients such as nitrogen and phosphorus [19,20]. Previous studies demonstrated that marine microalgae species are able to grow in wastewaters utilizing the available nutrients. For example, *Nannochloropsis* sp. (Eustigmatophycese) was able to grow in a medium containing low percentage of wastewater [21]. In another study, the marine haptophyte species *Pleurochrysis carterae* was able to grow in carpet-mill wastewater with low concentration of nitrogen and phosphorus [22]. These studies suggest that different types of wastewater might be used in microalgae cultivation. However, an optimization for high-lipid productivity is needed since the response of various microalgae species to different types and levels of wastewater is species-specific [23]. Many marine microalgae species are able to accumulate high quantities of lipids in response to unfavorable growth conditions; however, this is also associated with low biomass and lipid productivity [24,25].

Tertiary-treated wastewater is a valuable water resource in semi-arid and arid regions like Eastern Mediterranean. Particularly in Cyprus even though the majority of the produced tertiary-treated wastewater is used in agriculture and for the enrichment of surface and ground water resources, substantial amounts are discarded annually to the sea. Tertiary-treated wastewater also contains low amounts of nutrients and it could be a cheap and environmental friendly water resource that can be used as a supplement for the culture of marine microalgae. For the biodiesel industry, it is imperative to optimize lipid and biomass productivity without increasing the operational cost and the environmental impact of the biomass production process.

In the current study marine microalgae blooms were developed and tested for biodiesel production under laboratory conditions. The main questions addressed in this paper are: a) do microalgae communities established in blooms exhibit higher biomass and lipid productivity compared to their dominant species grown in monoculture conditions? and b) do tertiary-treated wastewater support high biomass and lipid production of the developed blooms and its dominant isolates? To answer these questions a two-step approach employed. In the first step, marine microalgae blooms were developed and evaluated for their biomass and lipid productivity. At the same time the dominant species from each bloom were isolated and evaluated for biomass, lipid productivity as well as for FAME composition. In the second step, different levels of tertiary-treated wastewater were used to evaluate the performance of the most promising bloom and its dominant isolate developed during the first stage of this study. Using this arrangement it was possible to underlie the importance of multi-algal diversity on the biomass and lipid productivity both in seawater and tertiary-treated wastewater.

2. Materials and methods

2.1. Sample preparation, marine bloom development, isolation of dominant species, and growth characteristic measurements

Seawater column samples were collected from coastal areas in Cyprus, i.e. the marina of Larnaca (LCA) and the fishing enclave of Liopetri (LP), at 50 cm depth, and filtered through a 50 μm-mesh plankton net (Fig. 1). Blooms were established after enrichment of one (1) liter of the filtered seawater with 1 ml of sterilized Conway enrichment medium (commonly Walne, after Walne [26]). The enriched seawater samples were then stored in a growth room chamber under controlled conditions (25 °C, light intensity of 80 μmol photon $m^{-2} s^{-1} m^{-2}$ and a 14:10 h L:D) in sterilized 2 L Duran bottles and with continuous mild aeration for 7 days until growth was observed.

During the bloom development phase, algal growth was monitored using optical microscopy and optical density of the culture at 680 nm (OD 680). In addition, biomass dry weight (BDW, $g L^{-1}$) was determined daily. Duplicate 10 mL samples were filtered through pre-dried and pre-weighed fiber filters that were dried for 12 h at 100 °C, cooled in a desiccator and weighed. Bloom productivity (BP) was measured for 7 days using the following equation

$$BP\ (g\ BDW\ L^{-1}\ d^{-1}) = \frac{BDW_{t_2} - BDW_{t_1}}{t_2 - t_1}$$

where t_1 and t_2 are the time intervals between early exponential and late exponential time, respectively.

Once the initial blooming stage was stable, samples were collected