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Coupling microalgal cultures with hydroponics: prospection for clean biotechnology processes

[1]Eduardo Caffagni de Camargo, [2]Carlos Aparecido Casali, [1,2]Ana Teresa Lombardi, [1,2]Maria Inês Salgueiro Lima

[1]Post-Graduate Program in Ecology and Natural Resources; [2]Department of Botany, Federal University of São Carlos, Rodovia Washington Luís, Km 235, CEP 13565-905, São Carlos, SP, Brazil.
Corresponding author: Eduardo Caffagni de Camargo e-mail: eccamargo11@gmail.com

Abstract

The inadequate disposal of liquid effluents from large-scale nutrient-loaded cultures, aiming at carbon dioxide fixation and/or bio products, can cause eutrophication in aquatic systems. In search of an ecologically satisfactory and economically viable solution to this problem, we tested algal cultures as fertilizers in the production of lettuce in hydroponic systems, employing the Nutrient Film Technique (NFT). Hydroponic commercial solution and modified LC Oligo medium were used as culture media for microalgae and hydroponic systems supply. The freshwater Chlorophyceae *Chlorella vulgaris* was chosen as experimental organism due to its high growth rate, competitivity and tolerance to a variety of environmental conditions. Lettuces attained highest average values of dry and wet mass when grown in normal hydroponic solution (control) and a 50% dilution of this medium, including microalgae. None of four different hydroponic systems showed significant differences between alga-free control and experimental treatments, indicating the absence of toxicity from *C. vulgaris* cultures. Despite the low lettuce production due to nutritional deficiency, as observed with LC Oligo medium, investment in *C. vulgaris* cultures as biofertilizers in hydroponics would be advantageous not only for the recovery of waste to generate usable biomass, but also for the reduction of nutrient concentrations in the effluent.

Keywords: Microalgae, Eutrophication, Hydroponics, Lettuce

Introduction

One of the topics discussed most nowadays is the worrying issue of global warming. Records from the Mauna Loa Observatory (NOAA, 2014) show that the global concentrations of carbon dioxide, the main anthropogenic greenhouse gas, increased from an estimated 280 ppm, in the pre-industrial period, to approximately 400 ppm in 2013. If CO_2 emissions continue unrestrictedly, we should expect a rise of more than 2 ºC in the average world surface temperature by 2100 (IPCC, 2013), which would have irreversible impact on the environment.

The culture of microalgae in photobioreactors has yielded promising results in the search for ways of removing atmospheric carbon dioxide (CO_2). Recognized for their high photosynthetic efficiency, tolerance to extreme environments and adaptability to intensive cultures, microalgae can be very efficient in the fixation of atmospheric CO_2 (Kurano et al., 1995; Benemann et al., 2003). Species from various genera, such as *Chlorella* (Chlorophyceae), stand out for this purpose mainly for their high growth rate. It is known that *Chlorella* sp., when grown in open pond reactors and fed with air containing 6-8% CO_2, can reach an efficiency of gas removal up to 50% (Kumar et al., 2010). For its strength and competitivity, *C. vulgaris* Beijerinck is one of the best-studied species, with great potential for practical application. Ohse et al. (2009) demonstrated the presence of high levels of protein in *C. vulgaris* biomass, which suggests its use in the economic sector of food supplementation. Also promising are the potential profits from algal biomass in biofuel production (Chisti, 2007; Singh and Gu, 2010), especially in view of the lipid production by several species of microalgae, including *C. vulgaris*, which contributes to the reduction of atmospheric CO_2.

In spite of the aforementioned benefits, large-scale algal cultivation, like any other industrial process, generates wastes, for which a proper destination is required if the activity is to be ecologicaly correct and clean. Besides dissolved and particulate organic materials (Lombardi and Vieira, 2000), the liquid effluent contains large amounts of unused nutrients, especially nitrogen and phosphorus, which can lead to eutrophication of rivers and lakes if discharged into the environment (Smith et al.,1999). The biosustainable solution to this problem would be to invest in new commercially viable production process, capable of consuming the effluent.

One possibility is to reuse the culture waste as a medium for hydroponics, a crop cultivation technique that does not require soil as substrate. Besides doing without the selection and preparation of an appropriate soil, hydroponics has many other advantages, such as the thermal control of the nutrient solution and plant root system, more effective utilization of water and nutrients, the smaller areas required for cultivation and the ease of mechanization and disease control (Jensen, 2002; Cometti et al., 2013).

Lettuce (*Lactuca sativa* L.) is among the most frequently cultivated vegetables in hydroponic systems. Advantageous for its easy handling and short production cycle (Gualberto et al., 1999), it is also regarded as a bio-indicator species in the detection of allelopathic substances released by other organisms. It is, indeed, an excellent choice for testing the possible existence of toxicity in secondary metabolites or other products synthesized by *C. vulgaris.*

Our goal was to assess the hydroponic production of *L. sativa* var. Grand Rapids employing two types of *C. vulgaris* cultures as nutrient growth media: one with standard laboratory medium, suitable for microalgae cultivation, and the other with a basic commercial hydroponic solution.

Materials and Methods

The experiments were performed in the Alga Biotecnology Laboratory and in the greenhouse of the Botany Department at Federal University of São Carlos, São Carlos (SP, Brazil). The two nutrient media tested were the hydroponic solution proposed by Hoagland and Arnon (1950) (Table 1) and the LC Oligo medium (AFNOR, 1980) (Table 2), both adapted. LC Oligo medium was chosen because it yields high *C. vulgaris* productivity, in terms of cell density, and costs less than other known culture media (Chia et al., 2013). The hydroponic Nutrient Film Technique (NFT), proposed by Cooper (1976), was adopted, since it has been proved to be a viable alternative for domestic wastewater treatment, capable of better nutrient concentration and assimilation than other conventional systems (Jewell et al., 1983).

Table 1. Chemical composition of the hydroponic solution, prepared in distilled water for laboratory cultures and in dechlorinated water for greenhouse cultures. Adapted from Hoagland and Arnon (1950)

Components	Concentration (mol L^{-1})
$Ca(NO_3)_2$	3.9×10^{-3}
KNO_3	3.96×10^{-3}
$MgSO_4$	2.82×10^{-3}
$(NH_4)H_2PO_4$	6.95×10^{-4}
Fe-EDDHMA ($C_{18}H_{16}N_2O_6FeNa$)	2.3×10^{-5}
Micronutrients(KSC Mix®)	6.15×10^{-6}

Table 2. Chemical composition of LC Oligo modified culture medium, prepared in distilled or dechlorinated water. Adapted from AFNOR (1980)

Components	Concentration (mol L^{-1})
$NaNO_3$*	9.4×10^{-4}
NH_4NO_3*	1×10^{-3}
$Ca(NO_3)_2 . 4 H_2O$	3.4×10^{-4}
$MgSO_4 . H_2O$	2.4×10^{-4}
K_2HPO_4	4.6×10^{-4}
$CuSO_4 . 5 H_2O$	1.2×10^{-7}
$(NH_4)_6Mo_7O_{24} . 4 H_2O$	4.8×10^{-8}
$ZnSO_4 . 7 H_2O$	2×10^{-7}
$CoCl_2 . 6 H_2O$	2.52×10^{-7}
$Mn(SO_4) . H_2O$ *	2.4×10^{-7}
H_3BO_3	9.8×10^{-7}
$FeCl_3 . 6 H_2O$	6.28×10^{-6}
$FeSO_4 . 7 H_2O$	4.48×10^{-6}
$C_6H_5FeO_7 .5 H_2O$	5.8×10^{-6}
$NaHCO_3$	3.58×10^{-4}

* Components not included in original medium

C. vulgaris culture

To adjust the concentration of hidroponic solution for microalgae growth, preliminary test cultures in four different dilutions of hydroponic solution (25%, 50%, 75% and 100%) made in distilled water was carried out in 250 mL Erlenmeyer flasks under controlled temperature (25 °C ± 1 °C) and light intensity (170 μmol m^{-2} s^{-1} PAR) conditions. The best result was chosen for the 20 L cultures grown in closed photobioreactors, with maximum values of 30 L capacity and 18 days duration. Artificial illumination was provided by 170 μmol m^{-2} s^{-1} fluorescent lamps (12:12 h light:dark photoperiod). Initial pH values for both media were adjusted to 7.0. The monitoring of photobioreactors was conducted on alternate days. Samples (2 mL) were collected for the determination of algal growth by cell counting in a Fuchs-Rosenthal chamber under the optical microscope (Leica, DM 1000, Germany).

The cultures were grown in two fed-batch systems (one with LC Oligo medium, the other with the best diluted hydroponic solution). Both inocula, with 6 L of nutritive medium and 20 mL of *C. vulgaris* culture (10^4 cells mL^{-1}), were divided into three portions of 2 L each on reaching the exponential growth phase. Each portion was used to inoculate 8 L of nutritive medium. The approximate initial cell density was 10^5 cells mL^{-1}. The cultures in two of the three photobioreactors, initially maintained under controlled laboratory conditions, received 10 L more of pure medium and were moved to a greenhouse under semi-controlled conditions. Once exponential growth was reached, the two 20 L cultures for each treatment were mixed, for subsequent use in hydroponics.

Hydroponic experiments

Hydroponics was performed in four different systems, in which the following media were used: LC Oligo with algae (OA), hydroponic solution with algae (HSA) and, as controls, pure LC Oligo without algae (O) and hydroponic solution without algae (HS).

Initially, 280 seeds of *L. sativa* were placed to germinate in phenolic foam cubes (2 x 2 x 2 cm), soaked with HS. This first stage was named the seed nursery (Figure 1a). The transplant to the main system was carried out five days after sowing. From then on, the solutions with/without algae, for the four treatments described above, began to be used for irrigation. The main hydroponic system (Figure 1b) consisted of three pipes for intermediate cultivation, with nineteen holes for the initial growth of lettuce seedlings, and three wider growing tubes with seven holes, to which only the seedlings that had developed best during the intermediate stage were moved. Eventually, 21 test organisms were produced in each of the four systems.

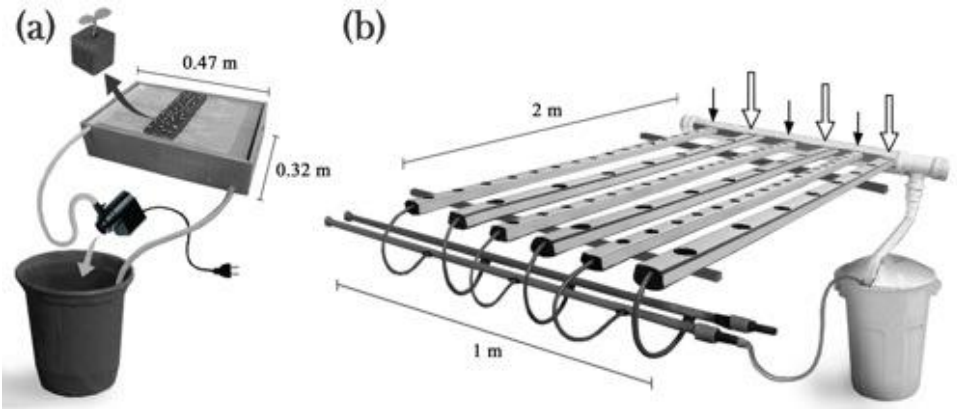

Figure 1. NFT hydroponic system used for lettuce production. (a) Seed nursery, with seeds sown in cubes of phenolic foam (dark arrow). The nutritive solution flows through the inclined channels on the lid of the box and returns to the bucket (light arrow), where it is stored and drawn back again into the system by a submerged motor pump; (b) Main system, with similar functioning, with three intermediate growth tubes (smaller black arrows), alternated with three growing tubes (larger white arrows), wider and with fewer holes for the plants. A single collecting pipe conducts the nutritive solution back to the tank

Dechlorinated tap water was added on alternate days to each hydroponic medium, to complete the volume to 40 L. Both media components were replaced as indicated by the difference in electrical conductivity between the appropriate standard value (550 µS/cm for OA and O, 950 µS/cm for HSA and 1500 µS/cm for HS) and the measured value. Controled pH was maintained at 6.0 to 7.0. A submerged motor pump (SarloBetter, S300), regulated by a timer was set to start irrigation at 6:00 am and stop at 8:00 pm, with alternating intervals of 15 min flow and 15 min stop, to create the laminar flow in each of the four systems during daytime. At night, irrigation occurred every 4 hours, lasting only 15 minutes. Average temperature in the greenhouse was 27.4 ºC, with 13 h day length (400 µmol m^{-2} s^{-1} PAR). The numerical counting of algal cells in the Fuchs-Rosenthal chamber under the microscope was also used to follow the proliferation of other species in the photobioreactors coupled to the hydroponic systems. These experiments lasted 35 days. After harvest, dry and wet masses of the aerial parts and root system of lettuces were determined on an electronic scale (Gehaka, BG 1000), accurate to 0.01 g.

Statistical analysis

The results obtained from the hydroponics experiments were analyzed statistically with R computer package (R Core Team, 2014). ANOVA, complemented by Tukey HSD test, was applied to parametric data, while the Kruskal-Wallis non-parametric test, followed by Dunn's test, was used in the absence of normal data (Zar, 1999). The program Origin 8.5 (OriginLab Corporation, Northampton, MA, USA) was used to elaborate graphs. The wet and dry masses of aerial parts and root systems of individual plants were compared with respect to production homogeneity within each of the hydroponic treatments and among the four tested systems.

Results and Discussion

The results for wet and dry biomass showed significant differences among the four systems (Figure 2) in relation to lettuces growth kept in LC Oligo medium and hydroponic solution. The 50% dilution of HSA was found to be the most favorable to algal growth in pretesting. This treatment resulted in greater development of *L. sativa*, with individuals reaching an average fresh weight of the aerial part of 45.86 g ± 23. This biomass was statistically equivalent to that obtained by cultivation in standard commercial medium HS (45.77 g ± 18.7). Although LC Oligo medium is favorable to the cultivation of *C. vulgaris*, it could not satisfy all the nutritional needs of *L. sativa*. On the other hand, *C. vulgaris* adapted well to the diluted hydroponic solution, which makes this medium a plausible choice for large-scale production of this microalgae with the advantage of lower cost than traditional microalgae culture media.

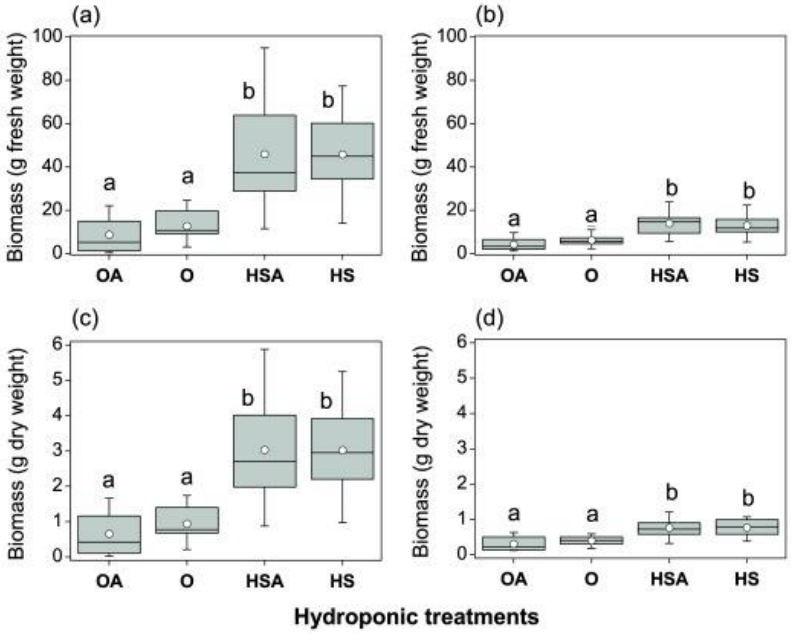

Figure 2. Average biomass of the lettuces for each hydroponic treatment: LC Oligo medium with algae (OA) and pure (O), hydroponic solution (diluted 50%) with algae (HSA) and pure (HS). (a) Fresh biomass of the aerial part, (b) fresh biomass of the root system, (c) dry biomass of the aerial part and (d) dry biomass of the root system

Statistical analysis indicated homogeneity of production among individuals under the same treatment, by comparing the three growing tubes of the system, which produced similar masses of lettuces. The biomass was statistically equal in the OA and O treatments, as it was in HSA and HS. Thus, we assume there was no interference of the *C. vulgaris* algal mass in the development of *L. sativa*. The absence of toxicity was indeed a beneficial result, since there have been reports of the production of growth inhibitors such as chlorellin by *C. vulgaris*, able to impede, for instance, the proliferation of other species of microalgae and zooplanctonic organisms (Fergola et al., 2007, Taub and Dollar, 1964).

The microfiltration of this alga-bearing hydroponic supply solution is therefore neither necessary nor recommended, since the continued presence of *C. vulgaris* can help, together with the lettuce, to reduce the concentration of left-over nutrients in the culture resulting from large-scale industrial production. The high adaptability of this microalga enables it to grow autotrophically, heterotrophically or even mixotrophically (Seyfabadi et al., 2011), depending on the conditions of the medium surrounding it. The main evidence of the potential of *C. vulgaris* in the cleaning of liquid effluents comes from reports of its important contribution to the treatment of urban wastewater, especially for the removal of nitrogen and phosphorus (Tam et al., 1994; Lau et al., 1996; Sreesai and Pakpain, 2007; Ruiz et al., 2011).

Microscopic analysis of culture samples showed that the HSA hydroponic solution resulted in the lowest levels of contamination throughout the experiment (data not shown). The contamination of large scale microalgal cultures is common, this being an inevitable problem in open cultures (Andersen, 2005). High contamination by *Chlamydomonas* spp. (Chlorophyceae) was found in LC Oligo medium, increasingly after the first half of the crop cultivation period. Similar results were obtained by de Schwarz and Gross (2004), who observed that algae of the *Chlamydomonas* genus are commonly present in closed hydroponic systems. Despite efforts to prevent contamination, the same authors highlighted beneficial effects offered by the algae, such as the prevention of anaerobiosis in the plant root system thanks to the oxygen produced by photosynthesis, and the release of plant growth-regulating hormones, such as auxins, that may be produced by microalgae (Mazur et al., 2001).

In an experiment that was the inverse of ours, Bertoldi et al. (2009) tested and demonstrated the viability of culturing *C. vulgaris* in wastewater from hydroponics, with the intention of incorporating unused nutrients into the algae biomass for further use in various applications, such as supplementation of fish food in aquiculture. The possibility of using organic effluents to promote microalgae culture is practicable in several areas and can help, apart from mitigating atmospheric CO_2, to lower the costs of biofuel production, which are still high compared to those of petroleum (Razzak et al., 2013).

Figure 3 summarizes the two best known and targeted goals of microalgal culture. Hydroponics may be proposed as a third goal, related to the reuse of waste generated by these cultures. Even if the plant biomass resulting from microalgae-produced hydroponics does not surpass that produced with the standard commercial nutrient solution, this activity remains a worthwhile initiative for its contribution to the reduction of eutrophication in surface waters. The economic benefits come not only from the sale of the vegetables, but also from the reduction in the costs of preparation of the hydroponic medium, given the utilization of a waste dispensed by industry. New toxicity tests will be needed for other species of microalgae, but the

principle of hydroponic application, together with projects for CO_2 fixation and the development of products from algal biomass, is a novel project that deserves investment.

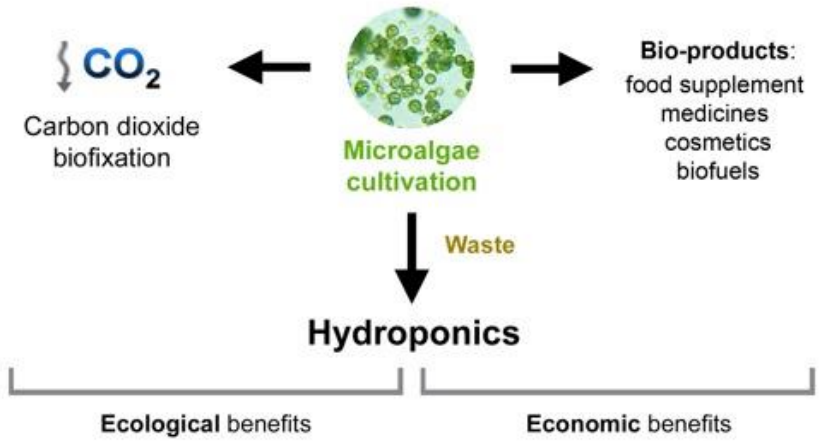

Figure 3. Diagram of possible environmental and economic benefits derived from the culture of microalgae. The utilization of the waste to hydroponics offers both of these: it avoids the eutrophication of the natural environment and provides a profit to the plant producer

Conclusions

The viability of the algal effluent produced with hydroponic solution (HSA) as a nutrient solution in the production of lettuce was clearly demonstrated. Moreover, its performance in terms of algal growth was similar to that with LC Oligo medium, proving that it can be advantageously coupled with photobioreactors in the cultivation of microalgae. Independent of the cultivation medium used, it was demonstrated that the presence of *C. vulgaris* in nutritive solutions did not interfere with the germination and development of *L. sativa*, which makes hydroponics a low-cost and ecologically viable destination for the waste from large-scale culture of this species of microalgae.

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