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Cultivation of *Spirulina maxima* in medium supplemented with sugarcane vinasse

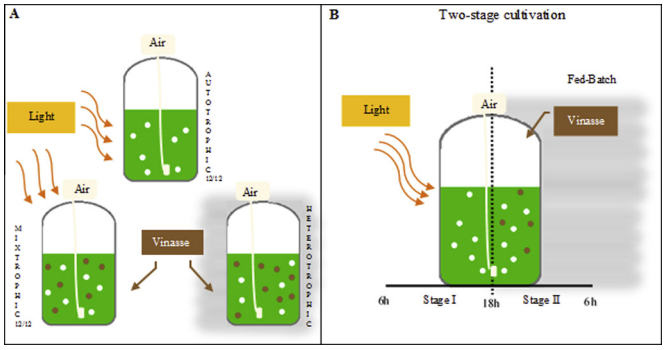
Raquel Rezende dos Santos [a,*], Ofélia de Queiroz Fernandes Araújo [a], José Luiz de Medeiros [a], Ricardo Moreira Chaloub [b]

[a] Escola de Química, Federal University of Rio de Janeiro, Av. Horácio Macedo, 2030, Centro de Tecnologia, Bl. E, Sala 201, Rio de Janeiro, RJ 21941-909, Brazil
[b] Instituto de Química, Federal University of Rio de Janeiro, Av. Athos da Silveira Ramos 149, Centro de Tecnologia, Bl. A Sala 532, Rio de Janeiro, RJ 21941-909, Brazil

HIGHLIGHTS

- Microalgae is a potential alternative for biologic treatment of sugarcane vinasse.
- Sugarcane vinasse is an organic carbon source for *Spirulina maxima*.
- *Spirulina maxima* grow under autotrophic, heterotrophic and mixotrophic conditions.
- Cyclic light-autotrophic/dark-heterotrophic strategy increases biomass productivity.
- Protein content in *Spirulina maxima* is appropriate for human and animal nutrition.

GRAPHICAL ABSTRACT



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ABSTRACT

The feasibility of sugarcane vinasse as supplement in growth medium of *Spirulina maxima* was investigated. The cell was cultivated under autotrophic (no vinasse, 70 μmol photons m^{-2} s^{-1}), heterotrophic (no light, culture medium supplemented with vinasse at 0.1% v/v and 1.0% v/v) and mixotrophic conditions (70 μmol photons m^{-2} s^{-1}, vinasse at 0.1% v/v and 1.0% v/v). These preliminary results suggested a cyclic two-stage cultivation – CTSC, with autotrophic condition during light phase of the photoperiod (12 h, 70–200 μmol photons m^{-2} s^{-1}) and heterotrophic condition during dark phase (12 h, 3.0% v/v vinasse). The adopted CTSC strategy consisted in three cycles with 75% withdrawal of suspension and reposition of medium containing 3.0% v/v vinasse, separated by autotrophic rest periods of few days between cycles. Results show an increase of biomass concentration between 0.495 g L^{-1} and 0.609 g L^{-1} at the 7th day of each cycle and high protein content (between 74.3% and 77.3% w/w).

1. Introduction

Microalgae are prokaryotic or eukaryotic microorganisms carrying out oxygenic photosynthesis found in both freshwater and marine environments, with a great potential for biotechnological applications (Stengel et al., 2011). Biofixation of CO_2 by microalgae potentially combines the steps of CO_2 capture from flue gases (replacing gas scrubbing technologies) and CO_2 utilization (through adequate biomass processing technologies), building a promising road for mitigation of greenhouse gas emissions. According to Chisti (2007), 1 kg of dry microalgae biomass utilizes about 1.83 kg of CO_2. Besides their high growth rates, they produce and accumulate biomaterials of commercial interest as pharmaceutical and nutraceutical products (Spolaore et al., 2006; Borowitzka, 2013) as well as renewable raw material for fuels

* Corresponding author. Tel.: +55 21 3938 7535.
E-mail address: raquel.c.rezende@gmail.com (R.R. dos Santos).

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and chemicals production (Chisti, 2007; Stengel et al., 2011; Araújo et al., 2015).

The ability of microalgae to proliferate over a wide range of environmental conditions is reflected in the huge biodiversity and heterogeneity in biomass composition. In fact, manipulation of the microalgae growth conditions can alter both the growth characteristics and the chemical composition of cells due to a reorientation of C allocation into lipids, carbohydrates and proteins under the conditions applied (Giordano and Ratti, 2013; Giordano et al., 2015). Although most microalgae grow under light condition with an inorganic nutrient source, some can grow in continuous darkness or simultaneously use an organic nutrient source (Stengel et al., 2011).

Many species of microalgae are able to effectively grow in brackish water because they are efficient in removing N and P from wastewater and, therefore, have the potential to play an important role in phyco-remediation. Several studies show high efficiency in the removal of inorganic nutrients, organic material, heavy metals and colour from effluents by microalgae (Pires et al., 2013). Some types of industrial or urban effluents have been tested with microalgae like domestic sewage (Sydney et al., 2011), piggery residues (Kuo et al., 2015) and dairy wastewater (Beevi and Sukumaran, 2014).

In this context, the sugar–ethanol industry generates large quantities of aqueous distillation residue generally known as vinasse or stillage. The characteristic of vinasse depends on the feedstock and route used for bioethanol production. Vinasse is usually acidic (pH 3.5–5), dark brown and with high organic content associated to chemical oxygen demand (COD) values from 50 g L^{-1} to 150 g L^{-1}. Bioethanol production from saccharose crop, starch crop and cellulosic material can generate considerable volumes of vinasse (1 L of ethanol leads, on average, to 9–14 L of vinasse) (España-Gamboa et al., 2011).

The main biologic alternative for large scale recycling of vinasse is fertirrigation. However, the systematic direct application of vinasse in the soil can lead to salinization, leaching of soil metals to groundwater, alkalinity reduction and phyto-toxicity increases (Christofoletti et al., 2013). Alternatives like anaerobic digestion and yeast production, among others, are yet under development and research (Laime et al., 2011).

The main aerobic system for vinasse treatment is the yeast and fungi production. At the same time, valuable products are also obtained such as biomass, organic acids and enzymes (Nitayavardhana et al., 2013). Although vinasse can be used as a culture medium or as an inexpensive media supplement to produce biomass, there are few reports concerning its use in culture of photosynthetic microorganisms.

Barrocal et al. (2010) analyzed the feasibility of using of vinasse from beet molasses fermentation as culture medium for growth of *Spirulina maxima*. More recently, Coca et al. (2015) evaluated the production of *Spirulina platensis* in medium supplemented with beet vinasse, as well as analyzed the influence of vinasse addition on biomass concentration and protein productivity. In Brazil, Marques et al. (2013) evaluated the feasibility of using the effluent of an anaerobic digester treating sugarcane vinasse for growth and lipid production by *Chlorella vulgaris* and Ramirez et al. (2014) evaluated the viability of producing *Scenedesmus* sp. using sugarcane vinasse as an alternative culture medium.

Considering that Brazil is the world's largest sugarcane producer and the world's second largest bioethanol producer, it is justified to investigate the application of sugarcane vinasse in cultures of photosynthetic microorganisms. In this context, the objective of this work was to analyze the feasibility of the use of sugarcane vinasse as supplement in culture medium for the growth of *Spirulina maxima*, focusing on the most suitable cultivation strategy and biochemical composition of the biomass obtained.

2. Methods

2.1. Sugarcane vinasse

The sugarcane vinasse was obtained from a Brazilian agroindustry located in the state of Rio de Janeiro. Vinasse was collected directly from distillation stills at about 95 °C and was immediately transported in 5 L bottles and stored at −4 °C until use. The vinasse was analyzed in order to assess its physicochemical and microbiological characteristics (Rand et al., 1976). The present vinasse exhibits the following characteristics: pH 3.11; total solids 22,802.7 mg L^{-1}; total dissolved solids 16,758.3 mg L^{-1}; total suspended solids 3244.0 mg L^{-1}; volatile solids 13,820.8 mg L^{-1}; biochemical oxygen demand 9060 mg L^{-1}; chemical oxygen demand 21,698 mg L^{-1}; ammonical nitrogen 3.41 mg L^{-1}; salinity 20 ppm; total reducing sugar 3909 mg L^{-1}; total protein 139 mg L^{-1} and number of yeast less than 10 CFU mL^{-1}.

2.2. Microorganism and culture medium

The cyanobacteria *Spirulina maxima* was kindly donated by Dr. Armando A. H. Vieira from Federal University of São Carlos, Brazil. The strain was maintained in 250 mL Erlenmeyer flasks containing 100 mL of AO medium (Aiba and Ogawa, 1977) sterilized by autoclaving at 121 °C for 20 min. The flasks were kept under constant agitation at 110 rpm (ETHIK TECHNOLOGY model 109-01 – 25 mm orbital motion), photon flux density of 20 μmol photons m^{-2} s^{-1} provided by fluorescent bulbs in 12:12 h photoperiod and room temperature of 25 ± 2 °C.

The AO medium was composed of $NaHCO_3$ (13.61 g L^{-1}), Na_2CO_3 (4.03 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $NaNO_3$ (2.5 g L^{-1}), K_2SO_4 (1.0 g L^{-1}), NaCl (1.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.2 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.04 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (0.01 g L^{-1}), $Na_2EDTA{\cdot}2H_2O$ (0.08 g L^{-1}) and trace metals solution (1 mL L^{-1}). Trace metals solution was autoclaved separately and was composed of $ZnSO_4{\cdot}7H_2O$ (0.22 g L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.81 g L^{-1}), H_3BO_3 (2.86 g L^{-1}), $Co(NO_3)_2{\cdot}6H_2O$ (0.049 g L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.039 g L^{-1}) and $CuSO_4{\cdot}5H_2O$ (0.079 g). Initial pH was adjusted to 9.5 with NaOH 1 M.

2.3. Culture conditions

2.3.1. Autotrophic, heterotrophic and mixotrophic cultivations

To obtain the inoculum, the cells were grown in 6 L bottles containing 5 L of AO medium. Culture bottles were maintained under room temperature of 30 ± 3 °C and 12:12 h photoperiod with a photon flux density of 70 μmol photons m^{-2} s^{-1} provided by fluorescent lamps. Cultures were gassed with filtered air pumped by aquarium compressors with 800 cm^3 min^{-1} airflow. The inoculum culture was used at the exponential growth phase. The photon flux density measurements were carried out using an integrated quantum meter LI-250A (Li-Cor Inc., USA) equipped with a cosine-corrected planar quantum sensor LI-190SA (Li-Cor Inc., USA). Sensor was located at the external surface of culture bottles.

The obtained inoculum was transferred to 6 L transparent bottles containing 5 L of AO medium with initial biomass concentration of 0.02 g L^{-1}, room temperature of 30 ± 3 °C and initial pH of 9.5. pH was allowed to vary freely during cultivation.

In order to carry out a preliminary investigation on the feasibility of *S. maxima* cultures in the presence of vinasse and the respective conditions for efficient growth, the cultivation was conducted under three different regimes: autotrophic (control), heterotrophic and mixotrophic. The vinasse was previously filtered and autoclaved.

In autotrophic condition, the cultivation was maintained in medium without vinasse and 12:12 h photoperiod with a photon