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Short communication

Protein production in *Spirulina platensis* biomass using beet vinasse-supplemented culture media

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ABSTRACT

Beet vinasse is a by-product from molasses fermentation factories, which is difficult to dispose of and involves a severe environmental concern. The present work deals with the valorisation of beet vinasse by means of the protein production in *Spirulina platensis* biomass. A preliminary study was firstly planned in batch flasks to analyse the influence of environmental factors on *S. platensis* protein content and protein productivity in beet vinasse supplemented culture media. Experimental conditions were selected to operate the photobioreactor that could provide a balance between protein content and cell growth. Then, *S. platensis* was cultured in an airlift tubular photobioreactor using mineral medium supplemented with beet vinasse (SVM media). The maximum cell concentration and protein productivity achieved in the tubular photobioreactor were $6.5 \pm 0.7\ g\,L^{-1}$ and $168 \pm 18\ mg\,L^{-1}\,d^{-1}$, respectively (with SVM $1\ g\,L^{-1}$ of vinasse). The addition of a higher vinasse concentration (SVM $2\ g\,L^{-1}$) led to biomass concentration and protein productivity values ($3.6 \pm 0.3\ g\,L^{-1}$ and $86 \pm 6\ mg\,L^{-1}\,d^{-1}$, respectively) closer to those achieved using unsupplemented mineral medium. Stable biomass concentrations were maintained during the continuous operation, thus demonstrating the ability of *S. platensis* to adapt to these culture media.

Keywords: Airlift bioreactor; *Spirulina platensis*; Sugar beet vinasse; Distillery wastes; Cyanobacteria; Single cell protein

1. Introduction

A major requirement in wastewater treatment is the need to remove nutrients, especially nitrogen and phosphorous, which can otherwise lead to risks of ecosystem damage and downstream eutrophication if wastewater is spilled into rivers and lakes. Microalgae are able to accumulate nutrients from wastewater and, therefore, have the potential to play an important remediation role (Ruiz-Marín et al., 2010). Simultaneously, the cultivation of microalgae can provide a useful source of biomass. It has been estimated that about 30% of the current world algal production is sold for animal feed application, i.e., aquaculture and poultry feed (Becker, 2007).

Spirulina platensis is a photosynthetic filamentous cyanobacterium often considered for biomass production due to its high cell growth rate, ease of harvesting and potential market as a human food supplement and animal feed. *S. platensis* is rich in a diverse range of active compounds such as protein, minerals, vitamins, pigments (phycocyanin and β-carotene) and polyunsaturated fatty acids (gamma-linoleic acid) (Cohen et al., 1987). Nutritional studies have shown that *S. platensis* contains essential amino acids in the proportions recommended by the WHO/FAO, except for methionine (Becker, 2007). Several reports related to the growth of *S. platensis* in mineral media have been published (Bezerra et al., 2011; Colla et al., 2007; Converti et al., 2006). Although it

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has been shown that *S. platensis* can metabolise both inorganic and organic carbon substrates (i.e., glucose, acetate) for mixotrophic growth (Chojnacka and Noworyta, 2004; Lodi et al., 2005), and ammonium and urea as alternative nitrogen sources (Matsudo et al., 2009; Sassano et al., 2010); the cultivation of *S. platensis* in complex media using industrial wastes, besides animal and livestock wastes (swine, cattle, poultry), is scarce (Markou and Georgakakis, 2011; Pittman et al., 2011). The use of waste and wastewaters to cultivate algae could reduce fresh water demand, nutrient costs and the need to waste remediation (Bhatnagar et al., 2011). Moreover, the use of an organic carbon source may allow a higher biomass concentration to be reached (Soletto et al., 2008). Vinasse, a by-product from molasses fermentation factories, is difficult to dispose of, and involves a severe environmental concern and a threat to watercourse quality (Coca et al., 2005; Mohana et al., 2009). The effluent is characterised by extremely high chemical oxygen demand (60–100 g COD L^{-1}) and biochemical oxygen demand (30–60 g BOD L^{-1}) (Mohana et al., 2009; Satyawali and Balakrishnan, 2008). It is, therefore, necessary to go through costly treatment stages before disposing of it. Apart from high organic matter content, vinasse also contains nutrients such as nitrogen, phosphorus and potassium. Vinasse has a relatively high protein equivalent, 18–22%, derived from the original nitrogen in beet molasses, mainly in the form of betaine. The mineral matter content is due to the presence of ions, mainly potassium, sulphate and chloride. Its composition suggests that the use of beet vinasse for *S. platensis* cultivation may be a value-added process which could reduce its environmental impact. However, there are very few published reports focusing on the cultivation of microalgae using vinasse (Montenegro-Ferraz et al., 1986; Travieso et al., 1999; Valderrama et al., 2002); while, to the best of our knowledge, there are no reports on *S. platensis* production. As the productivity and composition of cell biomass may differ according to species and culture conditions (Radmann et al., 2007), it is necessary to provide an understanding of the growth of *S. platensis* in cultures supplemented with beet vinasse. The aim of this work is to evaluate the production of *S. platensis* in mineral media supplemented with beet vinasse, as well as to analyse the influence of vinasse addition on biomass concentration and protein productivity in a vertical airlift photobioreactor.

2. Materials and methods

2.1. Beet vinasse

Vinasse was supplied by a beet sugar factory. It presented the following characteristics (on a wet basis): pH 6.0 ± 0.2, dry matter 58 ± 4% ww^{-1}, organic carbon 17 ± 4% ww^{-1}, total nitrogen 2.4 ± 0.4% ww^{-1}, organic nitrogen 2.0 ± 0.3% ww^{-1}, betaine 9.1 ± 0.5% ww^{-1}, phosphorous (as P_2O_5) 0.06 ± 0.02% ww^{-1}, potassium 6.4 ± 0.2% ww^{-1}, sodium 2.6 ± 0.2% ww^{-1}, calcium 0.6 ± 0.1% ww^{-1}, magnesium 0.1 ± 0.05% ww^{-1}, chloride 2.3 ± 0.2% ww^{-1}, colour 75 ± 5 absorbance units (a.u.) measured at 475 nm.

2.2. Microorganism

S. platensis, strain (SAG 21.99), was obtained from the culture collection of the University of Göttingen (Germany). Cells were maintained in the Schlösser culture medium (Schlösser, 1982) at 30 °C. This culture medium had the following composition (per litre): 13.6 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$, pH 9.3. The medium was autoclaved and, after cooling, 6 mL of PIV metal solution (750 mg Na_2EDTA, 97 mg $FeCl_3{\cdot}6H_2O$, 41 mg $MnCl_2{\cdot}4H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2{\cdot}6H_2O$, 4 mg $Na_2MoO_4{\cdot}2H_2O$) and 1 mL of Chu micronutrient solution (50 mg Na_2EDTA, 618 mg H_3BO_3, 20 mg $CuSO_4{\cdot}5H_2O$, 44 mg $ZnSO_4{\cdot}7H_2O$, 20 mg $CoCl_2{\cdot}6H_2O$, 13 mg $MnCl_2{\cdot}4H_2O$, and 13 mg $Na_2MoO_4{\cdot}2H_2O$) were added. PIV and Chu solutions were autoclaved separately before being added to the culture medium.

2.3. Experimental procedure

2.3.1. Flasks in batch

A preliminary set of batch cultivations were firstly carried out in flasks to select the operating conditions in the photobioreactor. Batch experiments were carried out in sterilised 250 mL Erlenmeyer flasks containing 100 mL of culture medium at 30 °C and 150 rpm. Each batch culture was inoculated with an initial *S. platensis* biomass concentration (dry weight) of 0.2 g L^{-1}. The concentration of inoculum was 10% (vv^{-1}). Cultures were illuminated by six fluorescent lamps (Osram F40CW, 40 W) for 16 h per day (8 h dark period). The influence of four environmental factors on cell biomass concentration and protein content was investigated through the runs performed in flasks. The variables were light intensity (24, 48 and 72 μmol photons m^{-2} s^{-1}), cultivation time (15, 20 and 25 days), vinasse concentration in culture medium (0, 2 and 5 g of wet weight vinasse per litre), and culture medium; the culture media being Schlösser medium at three different dilutions: undiluted, 75% Schlösser medium and 25% distilled water, and 50% Schlösser medium and 50% distilled water. The dilution of the Schlösser medium can save chemicals, but might also influence biomass concentration and composition. The initial pH was set at 9.2 ± 0.1 and allowed to vary freely during each test. At the end of each run, samples were collected to measure biomass concentration and protein content. Biomass productivity (mg L^{-1} d^{-1}) in batch cultivations was calculated from the equation $P_X = (X_t - X_0)/t$, where X_t is the biomass concentration (mg L^{-1}) at the end of each run, X_0 is the initial biomass concentration (mg L^{-1}), and t (days) is the cultivation time. Protein productivity, P_{PT} (mg L^{-1} d^{-1}), was calculated as the product of P_X and the protein content of dry biomass. The experiments were conducted in duplicate and the average data are shown.

2.3.2. Tubular photobioreactor

A vertical airlift tubular photobioreactor was used for the cultivation of *S. platensis* at 28–30 °C under a mean light intensity of 72 μmol photons m^{-2} s^{-1}. The bioreactor was composed of 16 horizontal PVC tubes. Each tube had a length of 1.2 m and 12 and 15 mm of inner and outer diameter, respectively, with a total volume of tubes of 2.3 L. The tubes were exposed to light from one side. The airlift system consisted of a compressor that injected 1 L min^{-1} of air at the bottom to recirculate the culture. A vertical PVC tube worked as a riser. A flask placed at the top of the photobioreactor was used as degasser and liquid reservoir. The total volume of the reactor was 3.55 L (tubes plus reservoir). A more detailed description of the photobioreactor can be found elsewhere (Barrocal et al., 2010). The operation in the photobioreactor began by filling up the reactor with mineral medium. An inoculum volume corresponding to 10% of the volume of the reactor was fed into it. The initial biomass concentration was 0.1 g L^{-1}. The reactor was operated in batch mode until a stationary biomass concentration