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Outdoor production of microalgae biomass at pilot-scale in seawater using centrate as the nutrient source

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ABSTRACT

In this paper the outdoor production of marine microalgae in tubular photobioreactors was studied, using real centrate from an urban wastewater treatment plant as the nutrient source. Experiments were performed modifying the centrate percentage in the culture medium (20, 30, 40 and 50%) and the dilution rates (0.2 and 0.3 day^{-1}) to study the phenomena taking place. Results confirm that marine microalgae can be produced under outdoor conditions using centrate as the nutrient source; at the same time treating it and recovering the nutrients contained in the centrate. The most efficient conditions for producing biomass were using 20% centrate and dilution rate of 0.3 day^{-1}, the biomass productivity was 15.62 $g_{biomass}{\cdot}m^{-2}{\cdot}day^{-1}$ and the photosynthetic performance was 0.54 $g_{biomass}{\cdot}E^{-1}$ and 2.6%. Regarding nutrients, centrate does not contain enough carbon to avoid carbon limitation thus the supply of carbon from flue gas is necessary. On the other hand, centrate is rich in nitrogen ($N\text{-}NH_4$), but only under optimal conditions is it efficiently fixed as biomass; otherwise it is stripped out to the atmosphere. Phosphorus is likewise only fixed efficiently as biomass under optimal conditions or it is lost by precipitation. It was observed a maximal nutrient removal capacity of up to 36.9 $mg_N{\cdot}l^{-1}{\cdot}day^{-1}$ and 5.38 $mg_P{\cdot}l^{-1}{\cdot}day^{-1}$. The population of the cultures was mainly composed of *N. gaditana* with conditions close to monocultures being achieved when maximizing the centrate percentage or reducing the set dilution rate. At any rate, the biochemical composition of the biomass produced did not show great variation, being rich in carbohydrates and proteins whichever the culture conditions given. The utilization of centrate to produce microalgal biomass allows to reduce the production cost and enhance process sustainability, reducing eutrophication, allowing the production of microalgal biomass for a range of low-cost applications such as feed or biofertilizers in addition to biofuels.

1. Introduction

Microalgae biomass is used in high value applications related to human and animal nutrition although they have also been proposed as a raw material for the production of commodities such as chemicals, biofertilizers and biofuels [1]. Producing commodities requires the production of large amounts of biomass, far higher than the present worldwide microalgae production of 20 $kt{\cdot}year^{-1}$ [2]. In turn, microalgae biomass production requires huge amounts of nutrients - from the elemental composition of microalgae, it has been concluded that 1.8 kg of CO_2, 0.10 kg nitrogen and 0.02 kg of phosphorous are necessary per kg of biomass. To supply CO_2, it is possible to use flue gases whereas nitrogen and phosphorus are usually supplied as fertilizers. However, the utilization of mineral fertilizers imposes a subsequent limitation on capacity, and reduces the sustainability of the biomass produced [3,4]. Using freshwater along with artificial fertilizers also increases the production cost to above 5 $€{\cdot}kg^{-1}$ [5,6].

Alternatively, microalgae can be produced using wastewater [7]. When using sewage and flue gases, the production cost is reduced by one order of magnitude (< 0.5 $€{\cdot}kg^{-1}$) [8]. However, when using wastewater, no pure cultures are obtained, with natural consortia of bacteria and microalgae prevailing under these conditions. The consortia composition largely varies as a function of water quality and operating conditions [9]. Wastewater is rich in carbon, nitrogen, phosphorus and other compounds required for microalgae production; these can contaminate rivers, oceans and underground aquifers [10] if not adequately removed. Therefore, wastewater can be used to replace the culture media prepared for microalgae production that before used fertilizers, increasing the sustainability of the entire process [11,12].

Because wastewater is typically freshwater, its utilization in microalgae production can limit the microalgae used to only freshwater strains. However, the utilization of marine microalgae strains offers

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advantages such as lower contamination risk, higher lipid content and valuable compounds compared to freshwater strains. For this reason, the coupling of marine microalgae production and wastewater treatment is of great interest. The best option for this coupling is to use centrate from anaerobic digestion as the concentrated nutrient source [13]. Centrate from the anaerobic digestion of activated sludge contains up to 1000 $mg_N \cdot l^{-1}$ and 30 $mg_P \cdot l^{-1}$ hence its depuration in wastewater treatment plants entails high cost and energy consumption. In a previous work, the production of *Nannochloropsis gaditana* was demonstrated using centrate from the anaerobic digester of a wastewater treatment plant as the nutrient source; although the supply of additional phosphorus helped to improve system performance [13]. Moreover, this concept was further demonstrated at the pilot scale under outdoor conditions [14]. In that work, the feasibility of using centrate as the nutrient source in seawater was demonstrated within the 15–30% range although experiments were only performed at one dilution rate in a narrow percentage of centrate - the influence of the dilution rate and a wider range of centrate percentages was not studied.

In this work, the continuous production of microalgae biomass in seawater using centrate as the nutrient source was studied. Experiments were performed in summer time, at two different dilution rates (0.2 day^{-1} and 0.3 day^{-1}); i.e., the daily flow rate of culture medium over the total volume of culture in the bioreactor. Whereas the percentage of centrate (i.e., the amount of centrate per liter of seawater, given as a fraction based on 100 equal parts) in the culture medium was modified (20, 30, 40 and 50%). System performance was evaluated in terms of dry weight biomass production/productivity and nutrient removal capacity. Additionally, an overview of the major strains prevailing inside the cultures as well as the biochemical composition of the produced biomass under study was performed. The objective was to determine the system's performance and the main factors limiting its efficiency as a prior step to the industrial development of low-cost marine microalgae biomass production for the commodities markets.

2. Materials and methods

2.1. Microorganism and culture media

The marine microalga *Nannochloropsis gaditana* Lubián CCMP 527 was selected because of its high growth rate and productivity under outdoor conditions [15]. Inoculum for the cultures was grown indoors at a pH of 8.0 controlled by on-demand CO_2 injection, at a controlled temperature (25 °C) in 5 l glass bottles at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ under continuous illumination and aeration at 0.2 $v \cdot v^{-1} \cdot min^{-1}$. The culture medium used for the inoculum growth was Algal medium (Bionova, Santiago de Compostela, Spain) prepared in seawater. After reaching the stationary phase, the cultures were transferred to 100 l outdoor bubble columns, under controlled pH (8.0) regulated by the on-demand injection of flue gas. Algal medium prepared in seawater was also used in these bubble columns. During the linear growth phase the cultures were finally transferred to the outdoor tubular photobioreactors in which mixtures of seawater and centrate at different percentages were used as the culture medium.

Centrate was taken directly from the wastewater treatment plant of Almería (Spain), after passing through the bed filter used to separate solids from the liquid fraction of the digestate leaving the anaerobic digestion of activated sludge produced from wastewater treatment. This centrate thus contained almost no solids yet was rich in ammonium and other compounds. The average centrate composition used during the experiments is shown in Table 1. The culture medium was prepared daily by supplementing natural seawater with centrate according to the centrate percentage set for each experiment. Additionally, potassium phosphate was added to achieve an N/P ratio of 5:1, which was previously demonstrated as allowing optimal culture performance [14]. Seawater was obtained directly from a seawater pumping station. No additional treatment or sterilization of the culture medium was performed prior to entering the reactors so as to minimize the production cost.

2.2. Photobioreactors and operating conditions

Experiments were conducted outdoors in a set of three fence-type tubular photobioreactors from May to September, with the daily mean irradiance and ambient temperature being 7.5 ± 0.5 $kWh \cdot m^{-2} \cdot day^{-1}$ (26 ± 3 $MJ \cdot m^{-2} \cdot day^{-1}$, 1200 + 170 $\mu E \cdot m^{-2} \cdot day^{-1}$) and 25.1 ± 4.5 °C. The tubular photobioreactors were built as previously described in [16]. Each reactor had a working volume of 340 l. The tubular reactors consisted of a vertically-arranged tubular solar receiver (125 m length and 0.05 m diameter) and a bubble column for heat exchange and O_2 degassing (1.92 m high and 0.25 m in diameter). A centrifugal pump (SE-150-M, Espa, Spain) was used to recirculate the culture through the reactor at 0.5 $m \cdot s^{-1}$. The inner-tube diameter was set at 0.05 m to maximize solar radiation capture. The reactors were oriented east–west and the distance between them was 1.6 m so as to minimize shadowing. The temperature during the day was kept under 30 °C by circulating the seawater through a heat exchanger. The flue gas was produced by a diesel-oil boiler connected to a compressor which was used to store the flue-gas for further utilization. At the boiler outlet, the flue-gas was cooled as necessary by passing it through a passive stainless-steel serpentine. Moreover, before being injected into the cultures, the flue gas was filtered through three sequential cartridge filters (1 μm) to reduce the particulate content in the gas stream. The average CO_2 concentration in the flue gas was 10.9 ± 0.5%. The air flow entering each photobioreactor was 0.25 $v \cdot v^{-1} \cdot min^{-1}$ (FR4L72BVBN flow meters, Key Instruments, USA), while the flue gas was injected as required to maintain the pH at 8.0, at a constant flow rate of 0.05 $v \cdot v^{-1} \cdot min^{-1}$ (FR4A41BVBN flow meters, Key Instruments, USA). Dissolved oxygen, pH and temperature values were measured online with DO and pH probes (5342 pH electrode and 5120 OD electrode, Crison Instruments S.A., Spain) connected to a MM44 control-transmitter unit (Crison Instruments, Spain). Data were logged in a PC control unit, allowing the monitoring and control of the culture parameters. The solar radiation the facility received was measured with a thermoelectric pyranometer connected to an AC-420 adapter (LP-02, Geónica S.A., Spain). The data logging system and the control software (DaqFactory 5.85, Azeotech Inc., USA) were designed and built by our research group.

The reactors were operated in semi-continuous, chemostat mode by adding daily fresh medium to the reactors for 4 h in the middle of the solar cycle and, at the same time, harvesting an equal volume of culture. Experiments were conducted at two different dilution rates, 0.20 and 0.30 day^{-1}. Culture medium was prepared directly by mixing the centrate with seawater pumped directly from the seawater pump station; four different centrate percentages (20%, 30%, 40% and 50%) were used. Percentage of centrate influences the amount of nutrients daily provided to the reactors whereas the imposed dilution rate also influences the microalgae biomass harvesting thus the final biomass concentration inside the culture at steady state. Fig. 1 shows the schematic process applied in these experiments. Experiments were performed in the three reactors concurrently and in triplicate providing the average values from the three reactors.

2.3. Biomass concentration and productivity

The biomass concentration was determined daily by measuring absorbance at 750 nm with a spectrophotometer (DR/4000 UV/Vis Spectrophotometer, HACH, USA). Spectrophotometric measurements were verified by dry weight determinations twice a week. The dry weight biomass concentration (C_b) was measured by centrifuging 100 ml of culture for 15 min at 9000 × *g* (Sigma Sartorius 4-15, Sartorius A.G., Germany) and freeze-drying over 48 h (LYOQUEST-55