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Utilization of centrate for the production of the marine microalgae *Nannochloropsis gaditana*

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ABSTRACT

In this paper, the production of the microalga *Nannochloropsis gaditana* using centrate from the anaerobic digestion of treated urban wastewater is studied. For this, semicontinuous cultures were performed indoors at laboratory scale, under controlled conditions, supplying seawater with different centrate percentages from a real wastewater treatment plant as the culture medium. It was demonstrated that *N. gaditana* can be produced using solely centrate as the nutrient source but only at percentages below 50%. Above this level, inhibition is caused by an excess of ammonia, thus reducing productivity. In the 30–50% centrate range, biomass productivity was 0.4 $g \cdot l^{-1} \cdot day^{-1}$, equal to that measured when using Algal culture medium. Moreover, the biochemical composition of the biomass was also equal to that measured when using Algal culture medium, with the protein content in the 30–40% d.wt. range; whereas the lipid content ranged from 20 to 25% d.wt. Under these conditions, phosphorus depuration from the culture medium was in the 80–90% range while nitrogen depuration was only between 20 and 40%, indicating an excess of nitrogen in the centrate with respect to phosphorus. In spite of this phosphorus limitation, in the optimal centrate range (30–50% in the culture medium), the cells performed under optimal conditions, removing up to 35 $mg_N \cdot l^{-1} \cdot day^{-1}$ and 5.7 $mg_P \cdot l^{-1} \cdot day^{-1}$, with quantum yield values measuring 1.0–1.3 $g \cdot E^{-1}$. By supplying additional phosphorus, it was possible to enhance productivity and increase nitrate and phosphorus depuration to over 80%. The use of centrate is confirmed as a useful method for reducing microalgae production costs while also increasing process sustainability, especially when using biomass for bioenergy applications.

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1. Introduction

Rising oil prices and global warming, associated with the burning of fossil fuels, have prompted a search for renewable, clean and carbon-neutral biofuels. In this scenario, microalgae have been proposed as a third-generation biofuel source given their high potential energy yield per hectare [4,19]. For this reason, considerable effort has been made recently to develop technologies for producing biofuels such as bio-diesel, bio-ethanol, bio-methane and bio-hydrogen from microalgae biomass [30,33]. However, the process has not yet been exploited industrially as the high cost of microalgae biomass production is still too great to compete in the energy field, especially given the limited availability and cost of nutrients [1]. When using clean water and artificial fertilizers, algae production costs are still very high, more than 5 €/kg of dry mass [1,22,25].

Nitrogen and phosphorus, in addition to CO_2, are the main nutrients required for microalgae production. Approximately 5 t of nitrogen and 1 t of phosphorus are needed to produce 100 t of microalgae biomass. The production of these compounds as fertilizers is limited as well as being associated with high energy consumption and resultant CO_2 emissions — indeed, to produce 1 kg of NH_3, more than 10 kWh of energy is required. Consequently, using fertilizers as the nutrient source reduces the sustainability of microalgae-based processes [16]. On the other hand, nitrogen and phosphorus can be obtained from effluents such as wastewaters. Because of this, microalgae production using wastewater as the nutrient source is a very promising alternative, which offers added environmental advantages [7,27,28]. As a result, microalgae can be produced from urban or animal wastewater using freshwater strains, at the same time helping to depurate the wastewater itself [3,9,24,26]. Microalgae production using wastewater, or other contaminated effluents, has additional advantages as microalgae are effective in removing organic matter, heavy metals and xenobiotics as well as inorganic nutrients [12,24,26] thus producing cleaner effluents with high dissolved oxygen concentrations. Moreover, the heavy metal concentrations found in wastewater are many times lower than the toxic levels for most microalgae strains [7]. Finally, wastewater depuration using microalgae consumes 0.52 MJ/m^3 compared to a value of 3.6 MJ/m^3 when using conventional systems, resulting in both economic and sustainability advantages (personal communication from Aqualia).

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Nonetheless, the utilization of wastewater limits biofuel production to freshwater microalgae strains even though using seawater strains is actually the most sustainable way to produce biofuels [36]. As an alternative, centrate from the anaerobic digestion of activated sludge produced in wastewater treatment plants can be used as the nutrient source to produce marine microalgae. There are two main advantages of using centrate: (i) the nutrient content is much higher than in wastewater, and (ii) the presence of aerobic microorganisms is scarce because they are produced under anaerobic conditions. Inside wastewater treatment plants the centrate is recirculated to depurate it, meaning higher energy consumption and greater cost. Utilizing centrate allows the nitrogen and phosphorus contained within it to be reused and reduces the number of stages required in the wastewater treatment plant, therefore reducing operating costs [7].

The centrate obtained from filtering the digestate (produced by anaerobic digestion) is the most concentrated stream of ammonium/phosphorus to be found in wastewater treatment plants. This centrate has already been used as the nutrient source to cultivate different microalgae strains such as *Chlorella* sp. *Chlorella vulgaris*, and *Nannochloropsis salina* [3,7,17]. Within the centrate, typical ammonia and phosphate concentrations range from 400 to 800 $mg \cdot l^{-1}$ and 20 to 60 $mg \cdot l^{-1}$, respectively. In addition to the concentration, the N/P ratio is also important because it determines the nutrient, which potentially limits the growth. This ratio should be close to the optimum nitrogen-to-phosphorus stoichiometry encountered in phytoplankton, which has been described as falling within the 8–45 range [13]. Centrate may also contain certain constituents that inhibit microalgae growth such as urea, organic acids, phenols and pesticides — at high concentrations these might limit the use of such effluents in microalgae production [15]. Consequently, research is needed to determine the optimal centrate percentage that can be mixed with seawater to support algae growth for whichever conditions apply. To examine this, a specific study looking at centrate from each wastewater treatment plant should be carried out to evaluate its subsequent use as a nutrient source in microalgae production.

The aim of this research is to determine the feasibility of producing *Nannochloropsis gaditana* microalgae using centrate from a real wastewater treatment plant located in Almeria, in which not only the productivity but also the quality of the biomass produced is analysed. To do this, experiments were carried out using Algal culture medium as the standard alongside culture media prepared by adding different centrate percentages to seawater. Mass balances were then performed to determine nutrient yields, and the optical properties of the biomass were analysed to determine the light-use efficiency of the cultures. The quality of the biomass produced was also analysed.

2. Materials and methods

2.1. Microorganism and culture media

The marine microalgae Eustigmatophyceae *N. gaditana* Lubián CCMP 527 was selected because of its high growth rate and productivity under outdoor conditions [31]. Culture inoculum was grown under controlled pH (8.0) and temperature (25.0 °C) conditions in a 0.5 l flask, at an irradiance of 150 $\mu E \cdot m^{-2} \cdot s^{-1}$, using Algal medium with 8 mM nitrate (Bionova, Santiago, Spain) in seawater [8]. This medium contains 22.4 $mg \cdot l^{-1}$ of phosphorus and 890 $mg \cdot l^{-1}$ of $NaHCO_3$, in addition to small amounts of iron, calcium, potassium, copper, etc. For the experiments, the culture media were prepared using natural seawater. The control culture medium was prepared by adding chemicals to natural seawater at standard concentrations corresponding to Algal culture medium. Experimental culture media were prepared by mixing natural seawater with different centrate percentages (10 to 80% v/v) taken directly from a real wastewater treatment plant located in Almeria, Spain. The natural seawater was pumped directly from the Mediterranean and filtered through 10, 5 and 1 μm pore-size filters prior to use. No additional treatment was applied to the seawater or culture mediums used. Centrate was obtained directly from the bed filter used in the wastewater treatment plant to separate the solids from the digestate liquid fraction, gathered after the anaerobic digestion of activated sludge produced from wastewater treatment. Therefore, this centrate did not contain solids and was rich in ammonia and phosphorus, in addition to other compounds. A complete analysis of the centrate used is shown in Table 1 while Table 2 shows a summary of the main compounds within the different culture media used.

2.2. Photobioreactors and culture conditions

Experiments were carried out indoors in four polymetil-metacrilate bubble-column photobioreactors (0.5 m in height, 0.09 m in diameter). The columns had a medium inlet as well as a harvest valve, together with a pH sensor input at the top. Air was bubbled up from the bottom of the column at 0.2 v/v min to agitate and remove the dissolved oxygen. The temperature was maintained at 20 °C by controlling the air temperature in the chamber within which the reactors were installed. To keep the pH within the optimum range (7.80–7.85), pure CO_2 was injected on demand into the air stream at 0.01 v/v/min. For this, pH 5330 probes and an R21 pH-controller from Crison were used. The reactors were artificially illuminated using 28 W high-efficiency fluorescent tubes (Philips Daylight T5). The illumination simulated the circadian cycle and two irradiance levels were assayed (300 and 500 $\mu E/m^2$ s). The irradiance value was experimentally measured as the mean value at 16 different positions; measurements were performed using a spherical SQS-100 Walz GmbH quantum sensor (Effeltrich, Germany).

Growth experiments were performed simultaneously in all reactors, which were inoculated with 10% of culture volume from the same standard inoculum. Following this, the reactors were operated in batch mode for 6 days, after which time they were operated in semicontinuous mode. Under these conditions, 25% of culture volume was harvested every day and replaced with fresh culture media. This was carried out using membrane pulse pumps that introduced fresh media into the reactors during the six central hours of daylight, at 0.11 $l \cdot h^{-1}$. This dilution rate (D) of 0.25 day^{-1} was previously defined as being optimal under these culture conditions using Algal culture medium (data not shown). Semicontinuous operation was repeated daily until the culture parameters remained constant, which meant for at least three days. In each experiment, the same culture conditions were assayed in two reactors, thus each experimental condition was assayed in duplicate. Measurements of the biomass concentration as well as the biomass and supernatant characteristics were performed by taking fresh culture from the reactor whereas the biochemical composition was determined from harvested biomass.

2.3. Biomass concentration, chlorophyll fluorescence, nutrient uptake and quantum yield

The dry-weight biomass concentration (Cb) was measured by filtering 50 ml of culture through 0.45 μm filters and drying it in an oven at 80 °C

Table 1
Composition of centrate obtained from a wastewater treatment plant used to prepare culture medium by mixing with seawater at different proportions.

pH			8.31
Conductivity			4.55 mmhos/cm 25 °C
Compound	Concentration, mg/L	Compound	Concentration, mg/L
Chloride	1093.76	Carbonate	24.00
Bicarbonate	646.77	Magnesium	19.00
Ammonium	615.48	Iron	0.39
Sodium	358.00	Boron	0.27
Potassium	102.00	Sulphate	0.22
Calcium	96.00	Zinc	0.09
Phosphorus	36.02	Copper	0.03
Nitrate	28.94	Manganese	0.02

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