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Outdoor production of *Scenedesmus* sp. in thin-layer and raceway reactors using centrate from anaerobic digestion as the sole nutrient source

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ABSTRACT

In this paper, we have studied the outdoor production of the freshwater microalgae *Scenedesmus* sp. in two open reactors (32 m^2): thin-layer (1.2 m^3) and raceway (4.4 m^3), using centrate from anaerobic digestion as the sole nutrient source. The aim was to recover valuable nutrients (nitrogen and phosphorus) from effluents in order to maximize biomass productivity. Experiments were performed in semicontinuous mode, modifying the centrate percentage within the culture medium. The optimal centrate percentage was 30% — above this value the culture's performance reduced, probably due to ammonium excess (above 122 mg l^{-1}). Using the raceway reactor, biomass productivity was 24 g m^{-2} day^{-1}, whereas using the thin-layer reactor, it increased up to 42 g m^{-2} day^{-1}. Nitrogen and phosphorus removal was demonstrated to be proportional to biomass productivity, with maximal values up to 38 mg_N l^{-1} day^{-1} and 3.9 mg_P l^{-1} day^{-1} being removed, respectively. Ammonium stripping was only relevant in the raceway reactor, due to its lower biomass productivity, with more than 40% of the inlet nitrogen being lost to the air. The thin-layer reactor also proved to be more photosynthetically efficient, with maximal values of 4.7% being measured. An economic analysis demonstrated that the thin-layer reactor allowed a reduction in the biomass production cost, in addition to utilizing waste as the nutrient source, with a minimum production cost of 0.9 €/$kg_{biomass}$ being estimated. In conclusion, it is possible to use centrate from anaerobic digestion as the sole nutrient source for the large-scale production of *Scenedesmus* sp. biomass thus reducing the biomass production cost by avoiding the use of expensive and non-sustainable fertilizers; while also obtaining returns from the treatment of this type of residue. Such a combination helps to increase the possibility of producing commodities, or biofuels, from microalgae by coupling their production to treatment processes.

1. Introduction

Microalgae are commercially produced for high-value applications such as nutraceuticals and for human consumption; overall production worldwide does not exceed 15,000 t per year and the production cost is in excess of 10 € kg^{-1} [3]. It has been proposed that microalgae might also be applied in low-value fields such as feed, biofertilizers and biofuels. However, to reach these markets, the production cost must be significantly reduced, down to 0.5 € kg^{-1}, while the production capacity has to be substantially increased, up to 10^6 t per year [5,10]. In order to reduce the microalgae production cost, it is essential to use highly-productive, cheap reactors whereas to increase the production capacity, cheap and available nutrients are needed.

Microalgae production cost is a direct function of the photobioreactor used and the productivity achieved [34,44].Whatever the photobioreactor technology used, the area and volume of the required reactor is an inverse function of its productivity; thus enhancing biomass productivity directly reduces the biomass production cost [1]. Although open raceways are the most-commonly employed technology, the utilization of open, thin-layer photobioreactors has been reported to increase biomass productivity by up to 55 g m^{-2} day^{-1} [26]; this is higher than values reported for tubular photobioreactors, which reach 45 g m^{-2} day^{-1} [1], and open raceways, achieving 30 g m^{-2} day^{-1} [29]. Even though both open and closed photobioreactors can be used, only open systems make it possible to reduce production costs below 10 € kg^{-1}. This is due mainly to the greater investment required for closed photobioreactors, which increases the depreciation cost. It is also due to the greater power consumption of closed photobioreactors, which likewise increase the operating costs [1]. It has been reported that, even at a large scale (up to 200 t y^{-1}), the minimum microalgae production cost achievable using closed tubular photobioreactors is

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12.6 € $kg^{-1}$with depreciation being the main contributing factor — making up as much as 78% of the total cost. Conversely, by using open reactors, both the depreciation and the operating costs are reduced. As a result, at the same scale and under the same conditions as those considered for tubular photobioreactors, the microalgae production cost reduces from 12.6 to 3.2 € kg^{-1} — a reactor cost reduction from 5 € l^{-1} for tubular photobioreactors to 0.1 € l^{-1} for raceway reactors [1]. Due to the lesser cost of raceway reactors, the main production costs become the raw materials and the utilities such as power consumption and water.

Once the reactor's productivity is optimized, the supply and cost of nutrients must be considered. Carbon, nitrogen and phosphorus are the main nutrients required for microalgae biomass production. Approximately 200 t of CO_2, 5 t of nitrogen and 1 t of phosphorus are needed to produce 100 t of microalgae biomass [5]. To supply CO_2, it is possible to use most industrial flue gases with no prior treatment although the supply mode must be optimized [15]. Nitrogen and phosphorus are usually supplied as fertilizers but their production is limited and it is associated with high energy consumption and resultant CO_2 emissions. Each kg of nitrogen produced generates approximately 2 kg of CO_2 — it has been reported that 45% of the effective energy input into microalgae cultures is in the form of nitrogen fertilizer [9]. Consequently, utilizing fertilizers as the nutrient source seriously limits capacity and reduces the sustainability of microalgae-based processes [21,49].To solve this problem, combining microalgae production with waste treatment has been proposed, in particular with wastewater treatment. Wastewater contains nitrogen and phosphorus in addition to other required compounds such as iron and manganese etc. — the majority of the compounds required to produce microalgae [14,33,36,38]. One of the effluent streams with the highest nitrogen and phosphorus content in wastewater treatment plants is digestate, obtained from the anaerobic digestion of sludge during the biological step of conventional wastewater treatment processes. This digestate is usually filtered to remove solids, resulting in a liquid solution or “centrate”, which is returned to the head of the process to be depurated. The utilization of centrate as the sole nutrient source for microalgae production has been previously reported [24,31,42,45,46]. In the centrate, ammonia and phosphate concentrations typically range from 400 to 800 mg l^{-1} and from 20 to 60 mg l^{-1}, respectively [2]. The optimal concentration of this centrate, which can be used as the nutrient source for microalgae production, has to be studied in each case.

The aim of this research is to compare the performance of two open reactors in producing microalgae biomass, a thin-layer reactor and a raceway reactor, using centrate as the sole nutrient source. For this, we selected the freshwater microalgae *Scenedesmus* sp. because of its robustness and high productivity. Experiments were performed in semicontinuous mode under real outdoor conditions to study the reliability of the process. Different centrate percentages in the culture medium were assayed, and their influence on the biomass productivity and nutrient removal rate was studied. The development of combined processes for microalgae production and waste treatment is a sustainable strategy to increase the portfolio of low-value microalgae applications.

2. Materials and methods

2.1. Microorganism and culture medium

The freshwater strain *Scenedesmus* sp. was used. Our group previously isolated this strain from freshwater used in greenhouse fertigation. *Scenedesmus* strains are widely reported on for outdoor production because of their tolerance to adverse conditions. Experiments were performed using Arnon medium (prepared using fertilizers instead of pure chemicals as the culture medium) as the standard, and mixtures of freshwater and centrate at different percentages (30%, 60% and 100%). The culture medium composition used is shown in Table 1.

Table 1
Chemical composition of Arnon medium and centrate used to prepare culture media by mixing with freshwater at different percentages. Concentrations expressed as mg l^{-1}. For heavy metals the maximum concentration allowed for water release into water bodies is included in parenthesis.

	Arnon	30% centrate	60% centrate	100% centrate
pH	7.5	7.7	7.4	7.0
COD	16.0	47.2	84.4	134.0
Carbonate	256.0	93.2	166.4	264.0
Bicarbonate	6.0	87.4	74.8	58.0
Sulfate	6.0	25.9	46.7	74.6
Nitrate	619.9	1.2	1.3	1.6
Chloride	78.9	129.1	238.3	383.8
Sodium	276.1	92.2	144.4	214.0
Potassium	325.0	38.6	75.2	124.0
Calcium	365.0	38.3	56.6	81.0
Magnesium	12.2	11.5	22.0	36.0
Phosphorus	39.3	11.3	21.6	35.3
Ammonium	0.0	122.6	244.1	406.2
Iron	5.0E+00	7.8E−01	5.5E−01	2.5E−01
Copper$^{(5.0E-01)}$	2.0E−02	7.2E−01	4.4E−01	6.0E−02
Manganese	5.0E−01	7.1E−01	4.2E−01	3.0E−02
Zinc$^{(5.0E-00)}$	6.0E−02	7.1E−01	4.2E−01	3.0E−02
Boron	4.0E−01	9.4E−01	8.7E−01	7.9E−01
Nickel$^{(1.0E-00)}$	1.3E−04	1.9E−03	3.8E−03	6.3E−03
Cadmium$^{(1.0E-01)}$	3.0E−05	4.5E−04	9.0E−04	1.5E−03
Lead$^{(5.0E-01)}$	9.9E−05	3.0E−03	5.9E−03	9.9E−03
Mercury$^{(1.0E-02)}$	7.2E−06	6.5E−05	1.3E−04	2.2E−04
TIC	52.4	20.0	38.9	64.2
TKN	140.0	95.6	190.2	316.3
TP	39.3	11.3	21.6	35.3

Centrate was obtained from a real urban wastewater treatment plant in Almeria (Spain), operated by Aqualia.

2.2. Photobioreactors

Two different open reactors were used, a thin-layer reactor and a raceway reactor (Fig. 1). Both reactors have the same 32 m^2area, and both have an aerated tank (1 m depth) where pH is controlled to 8.0 ± 0.1 by on-demand injection of pure CO_2 at 5 l min^{-1}, or air supplied at 50 l min^{-1} to remove oxygen. In the thin-layer reactor, the culture depth was 0.02 m, whereas in the raceway reactor it was 0.12 m. In both reactors, the channel width was 1 m. The total culture volume in the reactors was 1.2 $m^3$and 4.4 m^3 for the thin-layer and raceway, respectively. In the thin-layer reactor, the culture is pumped from the aerated tank to the first layer (0.4 m in height), using a low-stress centrifugal pump; then it is circulated by gravity at 0.2 m s^{-1}until it returns back to the tank. In the raceway reactor, the culture is circulated at 0.2 m s^{-1} using a rotating paddlewheel (1 m wide and 0.40 m high) actuated by an electric motor. A SCADA system monitors and controls the full operation of both photobioreactors. Environmental parameters such as solar radiation and ambient temperature are measured on-line. The culture conditions inside the reactors, such as pH (Crison 5333T + MM44), temperature (PT1000) and dissolved oxygen (Crison 9336 + MM44) were likewise monitored on-line. The actuation of pumps and valves within the system was also controlled by a SCADA system.

2.3. Culture conditions

Experiments were performed in semicontinuous mode in both open reactors used (thin-layer and raceway). For this, the reactors were initially inoculated with a 10% total volume of *Scenedesmus* sp. culture from a 3.0 m^3 tubular photobioreactor. Subsequently, the reactors were completed with Arnon medium prepared from fertilizers instead of pure chemicals, and were operated in batch mode for one week. After that, both reactors were operated in semicontinuous mode at 0.3 and 0.2 $day^{-1}$for the thin-layer reactor and raceway reactor, respectively. The dilution rates imposed were previously demonstrated as optimal