



Upscale of a laboratory rotating disk biofilm reactor and evaluation of its performance over a half-year operation period in outdoor conditions



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ABSTRACT

Biofilm-based microalgae cultivation techniques are promising technologies to overcome several issues of suspended cultivations, although only a few large-scale systems have been examined so far. In this study, a rotating biological contactor-based laboratory-scale Algadisk reactor of 0.39 m² was tested under low light intensity, and then scaled up to 15.9 m² and operated for 6 months in outdoor conditions in order to test its stability and biomass production efficiency with *Chlorella sorokiniana*. The highest biomass productivity observed in the lab-scale reactor on disk surface base was 3.2 g (m² day)⁻¹ with a 0.9 g mol⁻¹ biomass yield on light and 208 g kg⁻¹ dry weight content in biofilm. Due to pH crashes, extreme temperature variations, CO₂ limitation, and failure of disk rotation, the Algadisk pilot system showed varying biomass productivity from 0.5 to 8.4 g (m² day)⁻¹ on reactor footprint area. Also, biomass yield on light and biomass density remained lower than at laboratory scale. Nonetheless, a total of 7.4 kg CO₂ was fixed in the biofilm during the operating time. Despite the difficulties and the complexity of the system, over 20 weeks of continuous operation was achieved without the need of reinoculation.

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1. Introduction

More and more research focuses worldwide on the biotechnological and carbon capturing potential of microalgae. Microalgae play a huge role in CO₂ sequestration from the atmosphere in nature. This characteristic feature is also applied to capture CO₂ from industrial sources, for example, flue gases of combustion engines of power plants and biogas plants, thus reducing its effect on climate change while producing O₂ and biomass [1,2]. Due to their high diversity, applications of algae biomass vary from food and feed additives, pharmaceutical compounds, biofertilizers [3], biofuel production, including biodiesel, bioethanol, and biohydrogen production, [4], to wastewater treatment [5]. Additionally, microalgae production does not require arable land therefore it is not competitive with food and feed production [6].

Numerous cultivation systems have been developed and optimized for a more efficient biomass and/or compounds production, e.g., open pond systems, tubular photobioreactors, flat plate reactors, and

biofilm-based cultivation. Suspended systems are widely used techniques for large-scale algae cultivation; however, they entail several difficulties such as high water demand, surface attachment of algae, high cost of downstream biomass concentration, and large occupied area [7,8]. On the other hand, researches on biofilm-based algal cultivation techniques show promising results to overcome these drawbacks of suspended cultivation methods and could provide solution for economically feasible alga biomass production systems [9,10].

A wide range of biofilm reactors have been developed so far, for instance, vertical twin layer sheets and twin layer like systems [11–16], vertical rotating belts [17], algal turf scrubber [18], rotating algal biofilm reactor with spool harvester [19], and rotating disks [20]. Besides all the differences in the concept of their structure and operation, they all have one important quality in common: the high biomass content of the biofilm. The dry weight content of the growing biofilm is reported often around 100–175 g dry weight kg⁻¹ wet biofilm [9,19–23], in some cases it can reach up to 200 g kg⁻¹ [12,21]. These values are comparable with the solid content of centrifuged biomass from suspended cultures [24,25], albeit without the expenses of dewatering processes like centrifugation and/or additional chemical compounds for flocculation, which can reach up to 20% of the total cost of biomass production [26].

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Furthermore, high biomass productivities were achieved in biofilm reactors, ranging from 2 to 20–30 g (m²_{surface area} day)⁻¹ [11,16,20,22,23,27,28]. With changing the orientation of the surface from horizontal to vertical, the biomass productivity on footprint area can increase even 8 times higher compared to biomass productivity on surface area. [16,22,29]. The low water demand of cultivation and the separation of algae cells from the growth medium generate an easy and cost effective way to replace medium, in case of contamination or introduction of stress medium in order to produce high value added compounds. [15,27,30].

However, until now, the number of research projects conducted on biofilm reactors for microalgae cultivation is few; moreover, several systems only exist and operate in laboratory scale. To prove the benefits of the surface attached cultivation of microalgae, more outdoor, pilot-scale setups are needed with long-term continuous operation.

The objectives of this work are to (i) upscale the Algadisk design from laboratory scale to large scale, (ii) to examine and determine the biofilm production and dry biomass content in both cases, (iii) to continuously operate the pilot reactor in order to test the reformation of biofilm without reinoculation, and (iv) to establish/investigate whether using flue gas to provide inorganic carbon is a viable source in large scale.

The concept of Algadisk system, based on a rotating biological contactor with periodical harvesting, was first described and tested by Blanken et al. [20]. In the present study, a modified laboratory-scale and a pilot-scale Algadisk photobioreactor were constructed. Compared to the system used by Blanken et al. [20], the following features were changed: (i) orientation of the disks are parallel to each other, and (ii) the stainless steel disks are replaced by self-supporting but light-weight and inexpensive polyvinylchloride (PVC) disks. Moreover, (iii) the light source of the small-scale indoor system is placed above the disks, resulting in different light distribution and shading of disks. In case of the pilot reactor, (v) CO₂ was supplied from the flue gas of a biogas plant and (vi) natural illumination was used.

2. Materials and methods

2.1. Microalgal strains and precultivation

Two microalgal strains were used in the present study; for the laboratory-scale experiments, a *Chlorella* strain was isolated from Maros River, Szeged, Hungary, using the agar plate technique to obtain single colonies after enrichment of natural samples [31] and was preselected due to its high biomass productivity (data not shown). For the pilot experiments, *Chlorella sorokiniana* CCAP2011/8k (Culture Collection of Algae and Protozoa, Scottish Marine Institute, United Kingdom) was cultivated. *C. sorokiniana* CCAP2011/8k was selected for the outdoor, large-scale experiment as more data were available of its growth characteristics under high light intensities, based on Blanken et al. [20]. The cultures were pregrown in M8-a medium [32] supplemented with 33 mM urea as nitrogen source. The pH of the medium was set to 6.7–6.9 prior to sterilization. In the case of the laboratory-scale experiment, the cultivation flasks were kept in a growth chamber at the constant temperature of 25 °C and were illuminated with 150–200 μmol photons (m² s)⁻¹ by cool white fluorescence tubes (Polylux XLR F58W/840, GE Lighting, East Cleveland, USA) in a 16:8-h light:dark cycle.

The inoculum for the pilot reactor was cultivated in a 20-L flat plate photobioreactor in M8-a medium enriched with 16 mM urea. The reactor was illuminated by fluorescence tubes (Philips TLD 58W840, Amsterdam, The Netherlands), which provided 250 μmol photons (m² s)⁻¹ of light intensity. Additionally, the reactor was situated in a greenhouse and thus exposed to sunlight. The temperature of the culture medium was kept around 35 °C. In both cases, the cultures were concentrated to 100 g L⁻¹ cell density and used for inoculating the disks in the beginning of the experiments.

2.2. Laboratory-scale photobioreactor setup and operation

The Algadisk photobioreactor design was based on a rotating biological contactor (RBC) [33] and was further modified in order to enhance and examine microalgae growth [20, present study]. The main part of the laboratory-scale system (see Fig. 1) consisted of a polypropylene tank (75 × 35 × 20 cm in length × width × height) and of four disks, each 25 cm in diameter, placed parallel on one axle 15 cm apart from each other. The material of the disks was PVC that was roughened in advance with sandpaper of P80 grit size for faster initial biofilm attachment and stable regrowth of the biofilm after harvesting [34].

The reactor tank was connected to a buffer tank, which provided a consistent medium level in the reactor tank; continuous circulation and equal distribution of nutrients were supplied by a circulation pump placed in the buffer tank; flow rate was set to 10 L min⁻¹. Total filling volume of the system was 35 L. The M8-a growth medium with 33 mM urea was used for the cultivation of the algae. Disks were continuously rotated via a motor connected to the axle at 11 rpm. A half-cylindrical apparatus was designed for the distribution of light over the surface of disks; 6 cool white fluorescent light tubes (Polylux XLR F15W/840, GE Lighting, East Cleveland, USA; see wavelength information at www.gelighting.com) were used, in a day:night cycle of 16:8 h. Due to the orientation of disks and the light source, light intensity had deviations between the disks, with the average light intensity on the disk surface being 40 ± 15 μmol photons (m² s)⁻¹. In order to reduce biomass growth in suspension and enhance biofilm formation, the reactor tank was covered with a stainless steel sheet to decrease the amount of light penetrating the medium, i.e., the medium was kept in dark.

For the inoculation of the system, dense, precultivated cultures were poured onto the rotating disks and into the reactor tank. Regrowth of the biofilm after harvesting is promoted by remaining cells on the disk surface; thus, no reinoculation was needed during continuous operation.

The temperature and the pH of the medium in the system were monitored and controlled: temperature was maintained at 30 ± 1 °C, and pH was kept between pH 6.7–7 by sparging CO₂ gas into the buffer tank when reaching pH 7.

2.3. Pilot-scale photobioreactor construction and operation

The design of pilot Algadisk photobioreactor was based on the laboratory-scale photobioreactor (see Section 2.2), although modifications were applied to adapt the system to outdoor conditions, including reduction of material and operation costs. The schematic depiction of the pilot Algadisk is shown in Fig. 2. The Algadisk pilot reactor was positioned at a biogas plant in Almazan, Spain, with an east–west orientation, in such a way that both sides of the disk are illuminated over the course of the day. The system consisted of six units; each including the following parts: a disk, a tank with a transparent polycarbonate lid, and a rotating motor; these were all connected to a polypropylene buffer tank, in which all the sensors were placed, namely pH, dissolved oxygen, conductivity and temperature meter, and the gas sparger. The buffer tank and the units were connected with pipes and the media was continuously circulated in order to equalize the pH, nutrient content, temperature, and dissolved CO₂ content. The disks were made of PVC with a diameter of 1.3 m, and their surface was roughened by sandpapering prior to assembly. Rotation speed of the disks was set to 4 rpm.

The pH of the media was kept between pH 6.7 and 7, by adding flue gas to the buffer tank when the pH exceeded 6.9; sparging stopped when pH of the medium dropped back to 6.7; the control system was independent of day:night time. The flue gas originated from the CHP unit of the biogas plant and was cooled to ambient temperature and dewatered prior sparging into the buffer tank. This process also allowed for the reduction of the medium's DO content. Concerning sufficient CO₂

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