Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Detailing the start-up and microalgal growth performance of a full-scale photobioreactor operated with bioindustrial wastewater

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ARTICLE INFO

Keywords: Algae Biorefinery Air-lift photobioreactor Chlorella sorokiniana Technical water Ultrafiltration

ABSTRACT

In this study, a full-scale enclosed microalgal air-lift photobioreactor (PBR) module was operated using both defined and industrial wastewater (WW) media. In the effort to establish full-scale operation: a WW ultrafiltration system, two algal productions, and a harvesting microfiltration system were tested. Bioindustrial WW medium was treated with ultrafiltration and was demonstrated to be a viable microalgal growth medium at large scale; however, further treatment is needed for the removal of fecal coliform to meet drinking water standards. The fresh water mesophilic algae *Chlorella sorokiniana* was successfully grown on bioindustrial WW medium at suboptimal temperatures (< 25 °C) and natural lighting with peak specific growth rate (SGR) of 0.48 day⁻¹, consistent with lab-scale results from literature. Optical densities (OD) of the algae at 665, 680, and 735 nm were found to be viable proxies for cell number of *C. sorokiniana* grown outdoors with daily fluctuations, despite inherent differences in chlorophyll sensitivity at each absorbance wavelength. However, OD measurements at different reactor locations shown to diverge at the onset of growth. Greenhouse temperature and solar insolation were measured, where it was observed that the SGR did not considerably improve from higher solar irradiance during periods of lower temperatures. Finally, the viability of harvested cells after microfiltration was also examined, with a negative exponential correlation between cell death and the volume of remaining filter condensate ($R^2 = 0.9247$).

1. Introduction

Pilot-scale algae enclosed photobioreactors (PBRs) have demonstrated functionality for years: however, their production reliability and economic feasibility are still under consideration [1]. Wijffels and Barbosa [2] suggest that the development of biorefining techniques may be one avenue to create a sustainable and economical case for commercial algal production. One important economic factor limiting the feasibility of an industrial scale algae process is the cost of nutrients [3,4]. There have been extensive efforts to use wastewater (WW) nutrient sources to grow algae for biofuels to abate these costs. Nevertheless, despite the cheap supply of nutrients there are other challenges such as the content of toxicants in WW and competition with microbial and other microalgal species, which might limit the potential to use algae in a biorefinery concept. Microalgae high-value cosmeceutical, nutraceutical, and pharmaceutical products such as, phycobilins, fatty acids, proteins and pigments [5] cannot legally be produced from municipal WW [6]. In a full biorefinery concept, further processing can yield numerous potential products valorizing lignin and carbohydrates as fermentable substrates [7].

Industrial WW have many disadvantages as nutritional media for algal growth as they can be compromised by heavy metal concentrations, surfactants, and biocides [8]. Studies of algae treating industrial wastewater usually address heavy metal, pollutant, and N and P removal [9–11] with few studies exploring industrial wastewaters from mill effluent [12] and anaerobic industrial effluent [13,14] as a feedstock for algal growth. Wastewaters from biological processes such as fermentations, deemed "bioindustrial wastewaters" herein, are perhaps the most promising because of their nutrient content from spent cells and cellular debris. Meanwhile, some bioindustrial WW, not suitable for human consumption, can potentially be used as a growth medium alternative to incentivize industry to promote infrastructures diverting bioindustrial to be used as a media for high-value productions away from harmful waste streams.

Photon management in PBRs is well understood with the majority of research focused on stationary design considerations around latitude and weather [15]; however, little work has been done to incorporate both spatial light dilution and solar tracking into photon management

http://dx.doi.org/10.1016/j.algal.2017.04.030 Received 28 February 2017; Accepted 24 April 2017 2211-9264/ © 2017 Elsevier B.V. All rights reserved.







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Nomenclature		PBR PSII	photobioreactor Photosystem II
COD	chemical oxygen demand	PAR	photosynthetically active radiation
CFU	colony forming units	SGR	specific growth rate
LC ₁₀₀	lethal concentration 100 percent	TN	Total Nitrogen
OD	optical density	WW	wastewater

to increase areal productivity. Spatial light dilution can increase photosynthetic efficiency by positioning the PBR reactor rows to receive more diffuse sunlight than direct sunlight [16–18]. Depending on the PBR design and mixing, algae cells at the photoactive surface waste as much as 80% of the absorbed photons, during which cells in the culture interior become light deficient [19]. At high irradiance, algae must migrate between light saturated regions and darker regions to avoid photoinhibition and light limitation, respectively, to increase photosynthetic efficiency. This mechanism has also been engineered into systems as "temporal light dilution", "the flashing light effect", or "light-dark frequency", using mixing to reduce light deficiency and photoinhibition [20-22]. Overall, at large-scales, spatial light dilution ultimately occupies land surface area [23], increasing capital costs, while simultaneously compromising areal productivity. Typically, PBR panels placed in rows receive the majority of diffuse light from above the rows, where the amount of diffuse light reaching the entire reactor depends on the height and distance between panels [2,24]. Theoretically, if direct sunlight remained above reactors at solar noon all day, the spacing between rows or panels could be minimized and the reactor height could be maximized, while receiving diffuse and direct sunlight. However, Camacho et al. [25] claimed a positive relationship of cell death with water column height in air-lift reactors, in which the authors attributed the increased cell death concentrations to the longer period that a bubble has to attenuate to an algal cell, while rising through the water column, where the bubble then damages the microalgae at the water surface when popping.

The aim of this work was to elucidate the possibility of full-scale production of microalgae in a short light path PBR module utilizing spatial light dilution solar tracking, with simultaneous WW treatment in a biorefinery concept approach. Both production, WW pretreatment and post-production harvesting steps are included in the study. Key water-quality parameters of the bioindustrial WW algal medium were analyzed before and after ultrafiltration pre-treatment to investigate the WW as viable algal medium. Moreover, full assessment of algal growth on the WW medium inside full-scale outdoor enclosed PBR module was performed. Finally, we assessed the growth of the microalgae at extreme sub-optimal daily temperatures and irradiances.

2. Materials and methods

2.1. Algal strain and media

The freshwater algal strain *Chlorella sorokiniana* provided was used throughout all experiments. The *C. sorokiniana* was provided by Ecoduna (Ecoduna, Austria) in a bulk 40 L, 2.5 g L⁻¹ algal inoculum, which was used because of its demonstrated ability to grow inside an identical reactor and the availability of the dense inoculum. In Assay-I, the reactor module was supplied with 3 kg of NaNO₃, 0.5 kg of K₂HPO₄·3H₂O, and 1.5 L of nutrient solution (Compo, Germany). The nutrient solution contained 4% TN (1.0% ammoniacal nitrogen and 3.0% nitrate nitrogen), 3% available phosphate (P₂O₅), 6% soluble potash (K₂O), 1% calcium (Ca), 0.5% water soluble magnesium. Nutrients were supplied, as needed. A bioindustrial WW stream was used during experimental Assay-II and its characteristics were measured before and after ultrafiltration for key water quality and algal medium constituents (Table S1, Supplementary material). The same



Fig. 1. Light dilution in photobioreactor (PBR). Photo of light dilution design in Ecoduna PBR, where each panel can be seen receiving direct sunlight (left), design schematic of Ecoduna PBR; callout demonstrating algal growth, aeration bubbles, and the effect of sunlight reaching the panel (right). The arrow represents natural, direct sunlight reaching one face of every panel.

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