



Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors



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ARTICLE INFO

Article history:

Received 12 February 2016

Received in revised form 19 May 2016

Accepted 31 May 2016

Available online xxxx

Keywords:

Microalgae cultivation

Polyculture

Wastewater treatment

Offshore photobioreactors

Biofuel

ABSTRACT

Algae Systems LLC has designed and implemented a novel approach to wastewater treatment in which municipal wastewater is used to cultivate microalgae in modular, offshore photobioreactors (PBRs). At the Algae Systems plant in Daphne AL, this process was used to treat up to 50,000 gal/day of incoming raw wastewater. A combination of algae nutrient uptake, aeration by photosynthetically produced oxygen, and dewatering via suspended air flotation removed 75% of total nitrogen, 93% of total phosphorus and 92% BOD from influent wastewater. Offshore PBRs contained evolving polycultures of microalgae and associated heterotrophs, with community composition shifting based on the dynamic external and internal environment. During one year of operation, microalgae composition shifted from dominance of *Scenedesmus dimorphus* to a diverse polyculture dominated by genus *Chlorella*, *Cryptomonas* and *Scenedesmus*. “The more, the merrier” approach to species richness produced resilient communities, enabling efficient nutrient uptake due to niche complementarity and eliminating process downtime due to biological disruptions. The resulting biomass was suitable for fuel conversion via hydrothermal liquefaction due to consistent lipid content, low ash content, and consistent elemental composition. Biomass production rates ranged from 3.5 to 22.7 g/m²/day during continuous operation, with productivity predominantly driven by temperature and frequency of harvest.

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1. Introduction

Recent literature reviews propose that microalgae are a promising feedstock for sustainable biofuels. Microalgae can grow very rapidly, do not compete with food crops for arable land, and can utilize saltwater and wastewater [1–3]. The US Department of Energy currently targets production of 5 billion gallons per year of sustainable and affordable algal biofuels by 2030 [4]. Recent techno-economic analyses project a high present-day production cost for algal biofuels, showing a conservative baseline at \$18 per gasoline gallon equivalent [5]. Further cost reduction requires improvement are needed in all sectors of the technology, including algal productivity, downstream processing, and co-product valorization. In addition, reliable long-term and large-scale data sets are needed to accurately assess the status of the algae industry. Reducing production costs and improving the energy balance in

microalgal biofuel production is a challenge that will determine the long-term viability of algal biofuels as commercial fuel.

One apparent challenge of mass algae cultivation is the cost and availability of water and nutrients needed for algal growth. In order to replace only a small portion of fossil fuels, mass cultivation of algae would be limited by availability of commercial fertilizers, requiring either recycling of nutrients or utilization of alternative nutrient sources [6]. Many reviews strongly argue for use of municipal or agricultural wastewater as an efficient source of both nutrients and water [2,7,8] including a 2012 report regarding the sustainable development of algal biofuels by the National Academies [9].

Microalgae are effective at consuming both nutrients and carbon from wastewater [2]. Nitrogen and phosphorus can be almost completely removed by algae in suspension or in immobilized form [10]. In addition, daytime photosynthesis provides aeration and maintains high dissolved oxygen in wastewater allowing for effective biological oxygen demand (BOD) removal [11]. Aeration via algal photosynthesis eliminates energy demand for aeration, which typically account for approximately 50% of energy consumed during traditional activated sludge treatment [12]. Integrating wastewater treatment and algal biofuel production therefore provides cumulative benefits, eliminating the need for external water and fertilizer while providing efficient wastewater

Abbreviations: PBR, photobioreactor; HTL, hydrothermal liquefaction; PAA, peracetic acid; SAF, suspended air flotation; BOD, biological oxygen demand.

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treatment services, thereby offsetting a significant portion of biofuel production cost [13].

Commercial facilities generally focus on mass cultivation of single microalgal species with high lipid yields or other desirable traits even though the maintenance of monocultures has proven difficult and costly. Polyculture cultivation may increase productivity in microalgae via two main pathways: resource use efficiency and community stability (e.g. [14,15]). Multiple species, which occupy different functional niches, utilize resources more efficiently because of their different absorption spectra, nutrient requirements, uptake rates and overall physiology. This principle of “complimentary resource use” applies to nutrient sources such as nitrogen, phosphorus, silica, and carbon [16–20] but also light quantity and quality [21]. A community containing multiple species is also more stable under varying conditions when compared to disturbance-reduced productivity of monoculture [22, 23]. Designing optimal community composition using synthetic ecology principles where each co-habitant is carefully selected and planted into the community [24] requires extensive knowledge of the whole community and may be suitable only for a set of well-studied conditions.

Commercial open ponds containing only a single species of microalgae for long periods of time create an artificial state similar to an agricultural field and require constant maintenance to exclude the surrounding community [25]. Herbicides, pesticides, and other exclusion efforts are expensive and may pose a risk to the environment [26]. Functional polycultures are more robust and resilient and effectively lower the risk of culture crashes [27]. Use of wastewater implies use of polycultures and naturally evolving communities because wastewater cannot be completely sterilized in a practical and cost-effective way. Successful crop protection therefore necessitates use of theoretical concepts and ecological principles including polyculture cultivation.

Process optimization for production of algae biofuels and associated products requires access to robust, large-scale, and long-term data sets to account for environmental variation, scale-up effects, and process disruptions. This year-long study seeks to demonstrate coupled algae cultivation and wastewater treatment as a successful strategy towards bulk biomass production for large-scale development of algal biofuels. We have selected enclosed, offshore, floating photobioreactors (PBRs) as our cultivation platform because this system enables high CO₂ retention, eliminates evaporative losses, minimizes land use, and harnesses mixing energy and thermoregulation provided by the surrounding water body. We present a year-long overview of cultivation at large scale, addressing wastewater treatment effectiveness, biomass production, environmental variables, community composition, biomass composition, and key ecological aspects of large-scale algae cultivation in offshore PBRs. Despite the presence of uncontrolled environmental variables, we have developed methods to adjust and optimize the abiotic conditions inside the PBRs using harvest frequency, PBR depth, and inoculum size. We highlight several cultivation strategies optimized for both biomass production and wastewater treatment effectiveness.

2. Material and methods

2.1. Process overview

All wastewater treatment and cultivation was conducted at the Algae Systems LLC, demonstration plant, constructed in partnership with Daphne Utilities, in Daphne, AL. Raw municipal wastewater was redirected from the Daphne Utilities sanitary sewer and used immediately or held for up to 4 days in an on-site storage tank. Prior to PBR feeding, raw wastewater was pumped from the storage tank through a 70 µm filter (M2 Renewables Microscreen, model MS28) and disinfected using peracetic acid (PAA) at concentrations from 5–15 ppm. Disinfection performance was monitored using *Enterococcus* spp. abundance as a proxy. Even though the wastewater was never discharged directly to Mobile Bay, as a precaution, *Enterococcus* colonies were monitored with every batch of wastewater for compliance with

EPA standards for wastewater discharge (104 CFU per 100 mL). *Enterococcus* concentration was quantified using mEi agar plates (Hach 2811715), membrane filters (Hach 2936500) and filtration method (Hach method 1600). After filtration and disinfection, undiluted wastewater was pumped to the offshore PBRs and served as a cultivation medium for microalgal inoculum. At the end of the growth cycle, wastewater and algal biomass were pumped from the PBRs back onto land and the mixture was dewatered using Suspended Air Flotation (SAF, Heron Innovators). SAF separated the harvested mixture into 2 components: 1) algae slurry (~8% solids) which was processed using Hydrothermal Liquefaction (HTL) and 2) treated wastewater which was returned back to Daphne Utilities. The process is summarized in Fig. 1.

2.2. Floating offshore photobioreactors

Photobioreactors (PBRs) were constructed at the Daphne site from transparent, non-diffusive, durable polyurethane, using a radio frequency welder for assembly (model 12000PL from FIAB). Each PBR was 45.7 m long and 1.83 m wide, liquid depth was determined by the total volume inside the PBR and ranged from 5 to 25 cm. A constant gas headspace was maintained during cultivation to facilitate diffusion of gases and minimize opportunities for interior and exterior biofouling (Fig. 1). Modular PBRs were supported by lateral air filled hoses for buoyancy and PVC frames to maintain the desired shape. Each PBR was tethered to 2 pilings. The offshore field covered 0.5 acres of Mobile Bay, 12–48 PBRs were operated continuously. Wastewater was supplied and harvested via a subsea pipe system. Each PBR contained a single port for air and CO₂ addition to the headspace and two passive gas vents to prevent gas accumulation inside the PBR. The offshore field was fully automated and controlled via Programmable Logic Control (PLC). Wastewater feeding volume, harvest volume and air/CO₂ volume and ratio were predetermined each day based on experimental design.

2.3. Growth rates

A representative composite sample from each PBR was taken during each harvest. The sample was filtered onto GF/F filter and dried in the drying oven (40 GC lab oven by Quincy Lab) at 80 °C for at least 12 h. Harvest density was calculated in g/L. Growth rate (g m⁻² day⁻¹) was calculated based on harvested volume (L), harvest density (g/L), area of the PBR (m²) and the length of the growth cycle (days):

$$\text{growth rate} = \text{harvest density} \\ * \text{harvest volume/PBR area/cycle length}$$

2.4. Semi-quantitative evaluation of community composition

A composite sample of each harvested PBR was evaluated under the microscope (AccuScope 3025 microscope series with Infinity Analyze Imaging software, Lumenera Corporation). A drop of sample was placed under the microscope and 10 fields at 400× magnification were then examined. Detected genera were recorded as relative abundance (percentage of all cells detected, e.g. *Chlorella* spp. = 55%). A single microscope operator performed all microscopy scans to maintain consistency. The purpose of this community monitoring was not to provide detailed species counts and densities but to quickly evaluate major trends and patterns in the composition shifts. We refer to the term “algae” to describe both microalgae and cyanobacteria.

2.5. Biomass lipid content, ash content and elemental composition

Lipids were extracted via direct transesterification of dried algal biomass using methanol-acetyl chloride, followed by extraction in n-heptane. The heptane layer was removed by rotary evaporation

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