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A preliminary implementation of metabolic-based pH control to reduce CO_2 usage in outdoor flat-panel photobioreactor cultivation of *Nannochloropsis oceanica* microalgae

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ABSTRACT

A crucial challenge associated with high-density, commercial-scale, outdoor microalgal cultivation is maintaining pH stability without excessive use of CO_2 buffering. This includes a media-dependent, intracellular metabolic proton imbalance leading to the alkalization or acidification of growth media that results when algae consume, respectively, nitrate or ammonium ions. Feeding these two nitrogen sources can theoretically achieve balanced proton metabolism as well as pH control that is economically more favorable as compared to CO_2 buffering or acid/base addition. To accomplish this, a fed-batch nutrient feeding strategy must be adopted as a component of a model-based pH control system, particularly in the case of ammonium preference. This work represents a preliminary study of the challenges of implementing a nitrogen metabolism based open-loop pH control strategy in the challenging environment of an outdoor photobioreactor at the DOE-ATP^3 testbed facility in Mesa, Arizona during the high solar insolation period of summer 2015. The approach was limited to twice daily fed-batch addition while accounting for ammonium-N preference in a background of nitrate-based algae growth media. Despite these limitations, growth achieved for a photobioreactor operated based on predicted metabolic nitrogen demand (PND) was comparable to 'CO_2-on-demand' (CoD) for pH control. PND reduced CO_2 usage to <10% of CoD control, where the reduced buffering resulted in much greater pH fluctuations due to daily variations in light availability, and a much lower and more consistent media alkalinity (5.73–5.79 mEq/L) as compared to the CoD control (6.51–8.49 mEq/L). While this effort illustrates the utility of feed-forward model-based control, it further illustrates the need for far more sophisticated 'real time' monitoring and modeling to accommodate the dynamic outdoor conditions. A need for improved analytics for accurate closure of nitrogen mass balance is also indicated.

1. Introduction

Concerns of continued use of transportation fuels and other fossil-fuel derivatives in relation to national security, global economics, environment, and rural economic development have led to increased interest in renewable fuels and products from biomass feedstocks like oleaginous microalgae. Nevertheless, commercialization of the algal industry is constrained by high operating costs associated with growth control in large, outdoor raceway open ponds or photobioreactors (PBRs) [13]. Culture pH has the potential to influence a wide range of parameters within an alga production process; extracellular pH influences trans-thylakoidal membrane ionic gradients and electromotive forces in photosynthesis, the assimilation of nutrients including inorganic carbon, and even the surface charge/zeta-potential that mediate subsequent flocculation-mediated harvesting [9]. Because carbon is typically provided by CO_2 (or dissolved wastewater carbon), the major inorganic nutrient (and associated process cost) is nitrogen. Nitrogen sources can be provided in a range of states of reduction (e.g. ammonium, nitrate, urea), where the resulting nitrogen metabolism can have dramatic effects on the media pH through secretion or uptake of protons [15].

Small-scale culturing typically obscures the buffering role of CO_2 because the amount of gas supplied is in great excess of stoichiometric requirements, such that the CO_2 approaches an equilibrium condition that is amenable for algae growth [14]. In outdoor operation, pH control is often implemented by sparging CO_2 on demand (CoD) when the pH goes above a specified upper bound, and the resulting carbonate equilibrium acidifies the medium [1].

$$CO_2(g) \rightarrow CO_2(aq) + H_20 \leftrightarrow H^+ + HCO_3^-$$

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Because CO_2 suffers from poor solubility of gasses in warm water, the majority of the CoD may not transfer into the culture for consumption, thereby representing a significant process cost as well as greenhouse gas emissions.

Attempting to retain gas while allowing light penetration rapidly results in rapid heating of the culture and/or significant evaporative losses. Rather than utilizing CO_2 buffering, acids or bases can be added via feedback control based on pH sensor measurements. This approach is hindered, however, by the undesirable accumulation of inhibitory counter-ions and costs of acids—which are nearly as great as the nitrogen sources [6]. The conceptually simpler and more economical approach of formulating balanced growth-media by combining ammonium and nitrate nitrogen sources is limited due to the significant and preferential assimilation of ammonium before nitrate by algae [12], which lowers pH to the point of cell death.

In a circumstance where the algae cannot regulate its own nitrogen utilization to maintain pH, the alternative is to supply different nitrogen sources based on the metabolism that will result in the desired pH control. Metabolic modeling-based pH control faces many challenges that are even greater than control issues in a typical bioreactor due to the dynamic condition of outdoor culture; one must consider both predictable and unpredictable components of light, temperature, rainfall, and evaporation rates—all operating on different time scales. While incorporating sophisticated feed-forward modeling is a long-term goal that combines both the biology, physics, and environment, this initial manual implementation was focused on translating laboratory experience to outdoor conditions—including brutal temperatures exceeding 40 °C to gain further insights into the overall challenge. The DOE's ATP^3 (Algae Testbed Public-Private Partnership) facility at Arizona State University's (ASU) Mesa campus' AzCATI (Arizona Center for Algae Technology and Innovation) provided an opportunity for access to a large number of analytics and PBRs designed specifically for established algal cultivation methods. The transition from indoor experimental studies [14] to outdoor operation in this work represents a useful characterization of what challenges to anticipate in the next phase of moving the proposed metabolic pH control strategy from pilot to large scale systems.

In this work, a simple stoichiometric-based metabolic model for open-loop pH control was implemented based on daily predicted nitrogen demand (PND) and ammonium addition into nitrate-based algae medium in an outdoor pilot-scale, flat-panel PBR at AzCATI. The existing method of CO_2 on demand (CoD) was operated as the control. The observations and impact of this approach on operation and performance of these two different pH control operational strategies are discussed.

2. Materials and methods

2.1. Algae cultivation methods

2.1.1. Microalga strain

The marine microalgae *Nannochloropsis oceanica* sp. 0209 was obtained from the National Center for Marine Algae and Microbiota, Bigalow, Maine (formally known as the National Center for Culture of Marine Phytoplankton (CCMP). This alga was chosen as the model marine, lipid accumulating eukaryotic microalgae species for the comprehensive multi-site evaluation of algae performance for the U.S. Department of Energy ATP^3 program (www.atp3.org).

2.1.2. Growth media

Cultures were maintained in f/2 salt-water medium. Modified f/2 medium consisted of (per 1 L): 0.754 g $NaNO_3$, 0.067 g NaH_2PO_4, 0.0063 g $FeCl_3 \cdot 6H_2O$, 0.002 g $Na_2EDTA \cdot 2\ H_2O$, 1.8×10^{-4} g $MnCl_2 \cdot 4H_2O$), 6.3×10^{-6} g $Na_2MoO_4 \cdot 2H_2O$, 9.8×10^{-6} g $CuSO_4 \cdot 5H_2O$), 1.0×10^{-5} g $CoCl_2 \cdot 6H_2O$), 2.2×10^{-5} g $ZnSO_4 \cdot 7H_2O$), and 35 g Oceanic ™ Sea Salt Mix (Detroit, MI, etc.). A stock solution of 500 g/L NH_4Cl was prepared for pH adjustment for outdoor PBRs. Culture media were prepared by adding stock solutions to tap water inside the bubble-columns and PBRs.

2.1.3. Inoculation and cultivation scale-up

Microalgae were pre-cultured indoors and scaled up sequentially for inoculation of 60 L flat-panel PBRs. Microalgae were initially cultured in bubble-columns first at 0.1 L and then 0.8 L working volume. They were then inoculated at a minimum OD_{750} of 0.3 and cultured in logarithmic phase at 10–15 L working volume in 15 L flat-panel PBR with dimensions of $4' \times 4' \times 0.05'$. All indoor cultures were cultivated under continuous light at 200–300 μE m^{-2} s^{-1} light intensity supplied by polychromatic fluorescent light, while receiving 2% (v/v) CO_2 gas. Outdoor PBRs (Fig. 1) were then inoculated at a minimum OD_{750} of 1.0 (to avoid photo-bleaching) at 50 L working volume in flat panel PBRs with dimensions of $48'' \times 72'' \times 4''$ supplied by gas bubbling from the bottom via 1/16″-diameter holes drilled on the side of a PVC pipe. Prior to inoculation, the PBRs were thoroughly cleaned with a water jet, then filled with a 0.03% NaClO bleach solutions for at least 30 min contact time, and rinsed 3 times with water to remove residual chlorine levels as confirmed using test strips Pooltime™ 6-way test strips.

The temperature range for indoor bubble columns and PBRs was 22–28 °C based on laboratory room thermostat. For outdoor PBRs, culture temperature ranges of 24–29 °C were maintained by cycling 25 °C chilled water from a nearby Glacier® pool evaporative cooler model #GPC-250 through 1 cm ID stainless steel cooling coil with a 6 m of contact length per PBR. Temperature and pH of outdoor PBR cultures were measured using a handheld pH 100 ExStick® meter and probe. The pH probe was calibrated with temperature compensation using standard pH 4, 7, and 10 buffer solutions and was recalibrated every night to adjust for signal drift during cultivation. Data was acquired by YSI 5200 A multiparameter monitor, wirelessly transmitted, and recorded to a PC

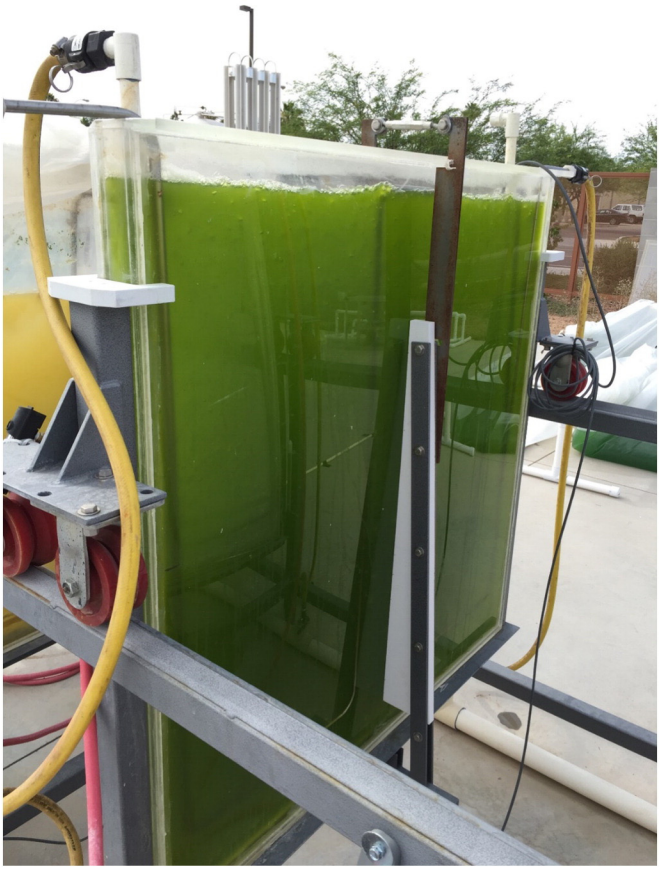

Fig. 1. Flat panel photobioreactor used for outdoor experimentation at the Arizona Center for Algae Technology and Innovation (AzCATI).