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Optimization of microalgae-sourced lipids production for biodiesel in a top-lit gas-lift bioreactor using response surface methodology

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ABSTRACT

Microalgae bioreactors that capture industrial carbon dioxide (CO_2) emissions to produce lipids for biodiesel are of significant interest. Sun-lit open raceways are generally considered the most economic method for mass cultivation, but the large physical footprint of these shallow systems can limit both industrial site availability and CO_2 transfer into the culture medium. To address these issues, a deep top-lit only, gas-lift bioreactor to culture microalgae and capture CO_2 was designed and investigated. The results show a three times increase in areal biomass and lipid productivities when compared to traditional raceways used in large-scale microalgae production. Operational factors exerting significant effects on areal biomass and lipid productivities were identified through Plackett-Burman experimental design as gas flow rate, feed gas CO_2 content, and dispersion height. By employing response surface methodology, models to predict areal biomass and lipid productivities were derived. The resulting desirability function was then applied to obtain the optimal combination of operational parameters that maximize lipid production per unit area occupied by the bioreactor, whilst keeping biomass production low to reduce downstream processing costs. The optimum operational parameters that fulfill the requirements of the optimization function resulted in areal biomass productivity of 32.1 $g_{dw}m^{-2}d^{-1}$ and areal lipid production of 198.4 $g_{Lipid}m^{-2}$.

1. Introduction

Use of industrial off-gas carbon dioxide (CO_2) to enhance the growth of microalgae, such as *Scenedesmus dimorphus,* which produce lipids suitable for transesterification into biodiesel [1] not only provides a useful biofuel, but CO_2 emissions can also be mitigated [2]. For this to occur on a large-scale, open ponds are still generally considered the most feasible option for microalgal cultivation [3]. These ponds take advantage of free sunlight energy, low mixing energy requirements, cost-effective construction and operation, and ease of scalability [4].

However, sun-lit open ponds need to be shallow at around 30 cm [5] due to light penetration limitations [6], which restricts their areal biomass productivity. A low areal biomass productivity means a large physical footprint, which could significantly restrict their location on industrial sites near to off-gas sources. Furthermore, shallow depths will limit gas-liquid contact times when off-gas is bubbled in. As a solution to increase areal biomass productivity and reduce the physical footprint, one-meter deep top-lit gas-lift bioreactors have been proposed [7]. These deeper bioreactors avoided the expense of sub-surface artificial lighting by using the gas-lift system and the greater depth improved gas-liquid transfer rates. The resulting areal biomass productivity for *S. dimorphus* of 60 $g_{dw}m^{-2}d^{-1}$ was significantly higher than the 5 to 45 $g_{dw}m^{-2}d^{-1}$ reported for traditional raceways and photobioreactors [8].

However, maximizing the level of suitable microalgal lipids, not overall biomass is key to biodiesel production. Irrespective of the type of bioreactor used, alteration of the biochemical composition of microalgal cells by manipulating growth simulators [9], bioreactor design [10], and media formulation [11] are known as effective methods to favor the lipid production. Growth conditions such as the availability of nutrients, including nitrogen [12] and sulfur [13], the concentration of CO_2 [14], pH level (basic environment [15], and acidic environment [16]), illumination area and photoperiod [17], light wavelength and intensity [18], temperature [19], and hydrodynamic stress [20] have been all reported as triggers of

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Nomenclature

A_{550}	Absorbance at 550 nm , cm^{-1}
A_t	Total occupied area of bioreactor, m^2
C_b	Biomass concentration, $g_{dw}L^{-1}$
D_i	Internal diameter, cm
D_o	Column diameter, cm
G	Gravitational acceleration, ms^{-2}
P_a	Areal biomass productivity, $g_{dw}m^{-2}d^{-1}$
P_{La}	Areal lipid production, $g_{Lipid}m^{-2}$
t_c	Mean circulation time, s
t_l	Light time, s
V_t	Total volume of bioreactor, m^3
X_1	Initial biomass density, $g_{dw}L^{-1}$
X_2	Gas flow rate, $Lmin^{-1}$
X_3	CO_2 content of feed gas (%), –
X_4	Dispersion height (Δh), cm
X_5	Media composition, –
Y	Response, –
β_0	Independent coefficient, –
β_{ii}	Quadratic coefficient, –
β_{ij}	Interaction coefficient, –
θ_L	Lipid content, –
ε	Light fraction

lipid synthesis in microalgal cells.

Some manipulation strategies, such as low pH or nitrogen deficiency [21], high irradiance [22], and high CO_2 concentration [23] resulted in an increase in lipid content, but at the expense of total biomass concentration. Therefore, in addition to an increase in lipid content, the relationship between lipid content and biomass concentration known as lipid production needs to be also considered.

The aim of this study was to systematically optimize the performance of a deep top-lit gas-lift bioreactor for a *Scenedesmus* sp. growth in terms of maximizing lipid production per unit area occupied by the bioreactor. This was done by evaluating the interactive effects of operational parameters, instead of using the traditional method of studying the effect of one individual parameter at a time. The aim is for the outcomes to help the economics of biodiesel production which simultaneously mitigates industrial CO_2 and minimizes the required site footprint. For the top-lit gas-lift bioreactor, therefore, various combinations of operational parameters including depth, aeration rate, CO_2 content of feed gas, nutrient concentration, and initial biomass density resulted in different growth conditions that affected algal cell biomass concentration and lipid content.

There have been many studies on the effects of individual parameters such as pH, salinity levels [24], medium composition [25], and CO_2 concentration [26] on the growth of *Scenedesmus* species. The complexity of interactive effects among the factors on the lipid productivity has not, however, been widely studied. To address this, applying a statistical experimental design method, response surface methodology, especially when there is a possibility of interaction among the large number of components, as an efficient strategy for the design of a microalgae cultivation system is proposed. Skorupskaite et al. [27] employed response surface methodology using central composite design to optimize biomass productivity and growth rate of *Chlorella* sp. Dhingra et al. [28] applied response surface methodology using central composite design to optimize biodiesel yield. Kirrolia et al. [29] employed response surface methodology using Box-Behnken design to optimize lipid content, carbohydrate, chlorophyll, protein and biomass production of *Chlorella* sp.

This current study was conducted in two steps. The first step, a factors screening phase, evaluated the influence of different parameters on algal growth and lipid accumulation through the application of a Plackett-Burman design. The second step, the optimization phase, was achieved by application of a response surface methodology. The optimum configuration of operational parameters to maximize lipid productivity was obtained, which was then verified with the top-lit gas-lift bioreactor.

2. Material and methods

2.1. Microalgae strain and inoculum preparation

The freshwater microalgae *Scenedesmus dimorphus* were acquired from the University of Texas, Austin collection (1237 UTEX collection). *Scenedesmus dimorphus* was selected due to its ability to grow under a wide range of CO_2 levels [26] and produce a higher lipid content compared to other microalgae, such as *Chlorella* sp. and *Chlorococcum* sp [24]. The stock culture was grown photoautotrophically and aseptically in Bold's Basal medium (BBM) [30] and incubated at 25 °C and 125 rpm. The stock culture was illuminated at 80 $\mu molm^{-2}s^{-1}$ on a photoperiod of 12 h light/12 h dark and supplied with Bold's Basal growth medium every three weeks.

2.2. The bioreactors configuration

The bioreactor used in this study was modified from previous study [7]. It consisted of a concentric draft-tube gas-lift column with an internal diameter (D_i) of 13 cm and height of 80 cm, secured in an outer column with a diameter (D_o) of 20 cm (Fig. 1). They were made from 5 mm thick, transparent plexiglas. The draft tube was located 5 cm from the bottom and gas was sparged in the draft tube as a riser section. The ratio of cross sectional area of riser to downcomer was 0.83 for the gas-lift reactor, which was within the range of values used by other studies [31]. The bioreactor had side ports at 5 cm and 50 cm from the base for taking samples. The depth of the bioreactor was varied by changing the dispersion height (Δh), which is the distance from the riser to the surface of the culture medium. Air mixed with carbon dioxide was sparged through a 10 cm diameter ceramic sparger with a mean pore size of 15 μm (Refractron Technologies Corp., NY, USA). The flow rate was controlled by using rotameters (±5%) (Omega Engineering Ltd., QC, Canada).

The anticipated application of the gas-lift system is for it to be installed, as with traditional commercial raceways, by burying it so that only the top surface is exposed. Therefore, the outside of the bioreactor was covered with a layer of black plastic sheet on top of a white plastic sheet to block light entrance from the sides. Light energy for the photosynthesis was provided only from the top of the bioreactor to simulate buried open systems on an industrial site, which is top lit by sunlight only. A 90 W circular grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) was used providing a 12 h light/12 h dark photoperiod. The photosynthetic active radiation at the surface of the culture was kept around 1000 $\mu molm^{-2}s^{-1}$ by changing the distance between the light source and culture medium surface. The surface