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Multi-parametric modelling and kinetic sensitivity of microalgal cells

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ABSTRACT

Mathematical modelling is a cognitive tool employed to describe the cellular response of microalgal cells to changes in nutrient inputs and other environmental factors. Currently, there exists no accurate model that simultaneously incorporates multi-parametric inputs such as carbon, nitrogen, phosphorus and light intensity as defining parameters for algae life. The main objective of this study is to develop mathematical models based on the defining four parametric inputs (i.e. carbon, nitrogen, phosphorus and light intensity) via Monod, Haldane, and Droop kinetics. These models were correlated with the growth data of microalgal cells in nutrient-saturated continuous cultures. Observed data did not conformed to the extended Monod and Haldane kinetics, but correlated to the extended Droop kinetics. The extended Droop kinetics (eDK) projected a similar trend in cell proliferation and response trajectory with the real-time experimental data. However, these model projections showed different transient dynamics in response to changes in the concentration of incoming nutrients in time-course simulations. Such differences suggest that the choice between Monod, Haldane and Droop kinetics to model the non-equilibrium dynamics of photosynthetic cells leads to widely divergent predictions of biomass proliferation. The mass transfer coefficient (k_l. a), is the most sensitive parametric input for biomass synthesis with maximum influence on the growth response trajectory.

1. Introduction

Kinetic modelling of microalgal cultivation is significant for designing proficient photobioreactors and calculating reactor performance. Mathematical models such as the Monod and Droop models are usually used to evaluate the specific growth rate of microalgal biomass in respect to the concentration of bioavailable nutrients present in the culture media. Several experimental designs have been explored on different microalgal species. In certain cases, the specific growth rate either defined by Droop or Monod model were considered as a function of one substrate (nitrogen, phosphorus or carbon) [1–5]. Other studies have employed the integrated form of Monod model to examine the specific growth rate of microalga as a combined function of both nitrogen and phosphorus concentration. Some of the studies, however, focused exclusively on the influence of light intensity via Monod model [6–8]. Microalgal growth kinetics have also been mathematically modelled via Michaelis-Menten or Monod equation [9], Haldane equation [10], and Steele equation [11].

The design of optimal input experimentation based on the above modelling approach to evaluate parametric sensitivity for microalgal growth kinetics is limited by the reactor design and research protocols. The Monod and Droop models were also developed based on the fundamental that the growth rate of a specific organism is limited by the organism's inaccessibility to a distinct parametric input. In effect, however, two or more parametric inputs typically limit its growth [12]. Thus, these models are not entirely accurate in estimating the specific growth rate of microalgal biomass whenever more than one parametric entity are limiting. Conversely, the influence of light intensity on microalgal cultivation is undisputable and should be considered as variable parametric input.

The consequence for existing kinetic models proposed to describe the correlation between microalgal specific growth rate and the parametric inputs have modified Monod or Droop model as a function of one or two limiting parametric factors. These proposed modifications have

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solely focused on describing a defined relationship between the microalgal biomass accumulation rate and one of the following parametric input: light intensity [6,7], nitrogen concentration [2], or phosphorus concentration [4]. Even though, empirical research by Yang [14] defines the specific growth rate of microalga as a function of three parametric factors (dissolved CO_2, light intensity and nitrogen concentration), there is need to prove the validity of an integrated Monod, Haldane and Droop model on the specific growth rate of microalgal cells.

Hence, this study aims to design a kinetic model that simultaneously incorporates all the operational variable parametric inputs that primarily influences microalgal cultivation. The specific objectives of this study are; (1) to develop extensions for existing kinetic models based on four parametric inputs (carbon, nitrogen, phosphorus and light intensity) and (2) to determine the sensitivity of the limiting parameters used in the developed models by designing peak trajectories for all input functions. This research provides novel data for input trajectory of multi-parametric functions influencing the specific growth rate and biomass accumulation of microalgal cells in a PBR system.

2. Materials and methods

2.1. Organism and culture medium

The microalga *Nannochloropsis oculata* obtained from AlgaeTech International Malaysia was used in this study. The cellular sample was stationed in an idle state overnight, then resilient cells were mildly glided and inoculated into Guillard F/2 marine enrichment media [12]. This enrichment medium is a concentrate of major nutrients, trace metals and vitamins diluted in 11 μm-filtered seawater to promote rapid process of cellular growth and biomass formation. The culture media was enhanced with 1 mL/L of minerals (sodium molybdate, ferric chloride, manganese chloride, cupric sulfate, cobalt chloride and zinc sulfate). To improve the survival rate of the microalgae strain, the aliquot was sub-cultured in a sterile 5 L Schott bottle containing the enrichment solution without silicate at 25 °C, under continuous surface illumination of 120 μmol·m−2·s−1. Green microalgal biomass was visible within a cultivation period of 7 days. These enriched cell cultures were mildly skimmed off and cultivation was scaled to 50 L acrylic glass columns designed photobioreactors. Cell cultures of *N. oculata* were cultivated in quadruplicates. The initial density of the inoculating strain cultivated in the PBRs was 9.80×10^6 cells/mL for each column. The PBR columns were closed to prevent contamination with dust. The PBR system ensured adequate provision of nutrient bioavailability for the microalgal cells to viably proliferate. With a cultivation media pH of 8.5, conductivity of 4.59 and salinity of 34‰ as determined by the BenchTop Water Quality Meter - IC860033, cultivation in the PBR was done under artificial lighting source (fluorescent light bulbs) providing a light irradiance of 120 μmol·m^{-2}·s^{-1} on the PBR surface (Cool White Plus by Philips, Netherlands), temperature of 25 °C and a light/dark cycle of 12:12, as suggested by Lopez-Elias et al. [13]. In order to resolve the mass balance derivations, parametric inputs from the present experimental design were applied in the model simulation as detailed in Table 1.

2.2. Operating mode

The nature and configuration of the device employed to circulate the microalgae suspension within the culture flux is vital in the design of an operative PBR. To achieve enhanced productivity and evade the expensive cost value of integrating mechanical fluid mixer; a bubble-driven aerated mixing technique via aeration pump (Secoh SLL-40, USA) was employed. Aside exacting circulatory flux within the bioreactor, the coupled air pump also serves to provide the essential gaseous environment required for the microalgae cells to proliferate. As a result, this hinders proliferating cells from settling; avoids the creation of temperature and pH gradients across the vertical axial of the PBR and enables efficient nutrients diffusion. It also removes photosynthetically synthesized oxygen capable of inhibiting photosynthesis at defined thresholds; transports CO_2 to the membrane of the cultivating cells and affirms that these cultivating cells experience alternating periods of light and darkness. However, it is important to note that excessive mixing can lead to cellular disruption and an eventual cell death usually results in the foaming conditions of the culture. For this reason, the aeration intensity dictated for the cultivation within the PBR system was 2 L·min^{-1}.

2.2.1. Gas retention coefficient ($\in_G$) and mass transfer rate ($k_l \cdot a$)

Several correlations have been proposed for calculating $k_l \cdot a$ in bubble reactors and different correlations are applicable under specific system settings such as the superficial gas velocity and reactor dimension settings [15]. The correlations propounded by Fair [16] to estimate $k_l \cdot a$ fits the operational settings of the microalgal cultivation system used in this experiment.

$$k_l \cdot a = 3.31 \frac{D_l \cdot \in_G}{{d_b}^2} \left[\frac{\mu_L}{\rho_L \cdot D_l} \right]^{\frac{1}{3}} \left[\frac{d_b \cdot \rho_L \cdot \mu_G}{\mu_L \cdot \in_G} \right]^{\frac{1}{2}}$$

where D_l is the molecular diffusivity of CO_2 in water (m^2 s^{-1}), $\in_G$ is the gas retention coefficient, μ_G is the dynamic gas viscosity (Pa s), ρ_L is the density of microalgae (kg m^{-3}), μ_L is the fluid viscosity of the cultivated biomass (Pa s), and d_b is individualized bubble diameter (m).

$\in_G$ was estimated by Shah [15] using Mersmann equation as:

$$\frac{\in_G}{\langle 1 - \in_G \rangle^4} = 0.14 \mu_G \left[\frac{{\rho_L}^2}{\rho_L - \rho_G} \right]^{\frac{1}{3}} \left[\frac{{\rho_L}^2}{\sigma \langle \rho_L - \rho_G \rangle g} \right]^{\frac{1}{4}} \left[\frac{\rho_L}{\rho_G} \right]^{\frac{5}{72}} \left[\frac{{\rho_L}^2 \cdot \sigma^3}{{\mu_L}^4 \langle \rho_L - \rho_G \rangle g} \right]^{\frac{1}{24}}$$

where c is the density of the influx gas (kg m^{-3}), g is the standard gravity constant (m s^{-2}), and σ is the water interfacial tension (Nm^{-1}). Dynamic gas viscosity (μ_G) is primarily a function of temperature. The dynamic viscosity of CO_2 (Pa s), is computed using Sutherland's formula [17]:

Table 1
Parameter estimations from the present experimental design.

Design factors	Value	Design factors	Value
Illuminated surface area, A_R - PBR (m^2)	1.32	Reactor diameter, D_R (m)	0.20
Reactor volume, V_R (m^3)	0.06	Reactor height, H_R (m)	2.00
Diffuser shape	Disc	Concentration of inoculum biomass, $[X]_i$ (kg·m^{-3})	1.01
Initial nitrogen concentration, $[N]_0$	20.2% w/v	Initial phosphorus concentration, $[P]_0$	5.0% w/v
CO_2 influx rate, R_{co_2} (m^3·d^{-1})	0.72	Diffuser dimensions	105 mm *diameter* 14 mm *thickness* 8 mm *tube port*
CO_2 volume, V_{co_2}	2%		
Gas pressure, P_{air} (atm)	6.5	Bubble form/size, d_b	Fine bubble 2 mm *diameter*
Fluid mixing rate (m^3·d^{-1})	14.4		