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Dynamic modelling of mixotrophic microalgal photobioreactor systems with time-varying yield coefficient for the lipid consumption

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HIGHLIGHTS

- A mathematical model to predict biomass and lipid production is proposed.
- Experiments were performed based on optimal experimental design.
- Time-varying yield coefficient is introduced to explain lipid consumption.
- Model validation was performed and good agreement with experimental results.

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ABSTRACT

Microalgae have been suggested as a promising feedstock for producing biofuel because of their potential for lipid production. In this study, microalgal photobioreactor systems under mixotrophic conditions were investigated, for the purpose of developing a mathematical model that predicts biomass and lipid production. The model was developed based on the Droop model, and the optimal input design using D-optimality criterion was performed to compute the system input profile, to estimate parameters more accurately. From the experimental observations, the newly defined yield coefficient was suggested to represent the consumption of lipid and nitrogen within the cell, which reduces the number of parameters with more accurate prediction. Furthermore, the lipid consumption rate was introduced to reflect the experimental results that lipid consumption is related to carbon source concentration. The model was validated with experiments designed with different initial conditions of nutrients and input changes, and showed good agreement with experimental observations.

1. Introduction

Microalgae are photosynthetic microorganisms, which can produce large amounts of lipids that can be used directly as high value bioactives, or be used to synthesize biodiesel. As worldwide interest in alternative fuels has increased, the attention to microalgae as a feedstock for biodiesel is growing recently. The lipid contents in microalgae range from 15 wt% to 77 wt%, depending on the species or culture conditions (Yusuf, 2007). Although the lipid production rate in microalgae is strain dependent, it has several advantages as a feedstock for biodiesel, including high growth rate and the ability to produce large amounts of lipid (Miao and Wu, 2006; Minowa et al., 1995; Xiao et al., 2013). However, biodiesel from microalgae is not economically competitive compared to biodiesel from conventional plant sources or petrodiesel (Yusuf, 2007). For economic competitiveness, it is necessary to improve the cell's growth rate or productivity of lipid by operating the bioreactor at optimal conditions. The process optimization and metabolic engineering are two complementary approaches to enhance productivity of bioreactors and a dynamic model is an essential element in both approaches (Song et al., 2013). A mathematical model that describes algal growth and lipid accumulation is useful for predicting the productivities of microalgae, optimizing the cultivation conditions, and scaling up for industrial production.

Microalgae can be grown under autotrophic, heterotrophic, or mixotrophic growth conditions. Compared to autotrophic cultivation, heterotrophic and mixotrophic cultivations allow some microalgae to accumulate much higher lipid content, as well as to provide high biomass productivity (Miao and Wu, 2006; Li et al., 2007; Chen, 1996). In the case of *Chlorella protothecoides*, heterotrophic cultivation with glucose as an organic carbon source results in four times higher lipid contents than autotrophic cells, and the color of heterotrophic cells (yellow) differs from autotrophic cells (green) (Miao and Wu, 2006). Under autotrophic growth conditions, growth is limited by light availability; the growth rate is

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reduced during night or in dark areas. However, under mixotrophic conditions, microalgae can use organic carbon sources to support their growth even in the night or dark areas. It was reported that only few microalgae can be cultivated mixotrophically, and among them are freshwater flagellate *Haematococcus pluvialis*, *C. protothecoides*, and *Ochromonas minima* (Liang et al., 2009).

Lipid productivity of microalgae is also influenced by nitrogen. The nitrogen deficiency reduces cells growth rate, but the content of the lipid increases (Pruvost et al., 2009; Mairet et al., 2011). Therefore, there is a trade-off relationship between growth rate and lipid productivity, and how to increase lipid content while maintaining cells growth properly by manipulating nitrogen concentrations is an important optimization problem.

In order to explain cell growth in bioreactor systems, a large number of models have been proposed in the literature. Among them, the Monod and Droop models are most widely used in control applications, since they are simple enough to apply model-based control strategies. For algal systems, the Droop model explains cell growth as a two-step phenomenon; the uptake of nutrients first occurs in the cell, and then intracellular nutrient is used to support cell growth (Droop, 1968). Recently, models including lipid fraction have been presented as the interest has focused on the lipid production in the microalgae. A modified model based on the Droop model is presented, to predict the neutral lipid fraction under nitrogen stress (Yang et al., 2011; Mairet et al., 2011). A lipid production model considering the simultaneous effect of carbon and nitrogen on the growth rate is also reported (Surisetty et al., 2010; De la Hoz Siegler et al., 2011).

In this study, a mathematical model that predicts the cell growth rate and lipid productivity under mixotrophic conditions varying nutrient conditions (glycine and glucose) and light intensity with *C. protothecoides* as a strain was developed. The experiments were performed based on the optimal experimental design and model parameters were estimated. From the experimental results, a newly defined concept of time-varying yield coefficient was applied and obtained better prediction performance with less number of parameters. The lipid consumption rate is also introduced to the model. Finally, the model was validated with the experiments which were designed with different initial and input conditions.

2. Methods

2.1. Microalgae and media composition

C. protothecoides, UTEX B25 (UTEX Culture Collection of Algae, Texas), were cultivated under mixotrophic conditions. *C. protothecoides* maintained on agar plates with proteose medium were subcultured in a flask with 150 ml culture media, and incubated at 25 °C and 200 rpm for 96 h. The composition of the culture media was as follows: KH_2PO_4 (2.8 g/L), K_2HPO_4 (1.2 g/L), $MgSO_4{\cdot}7H_2O$ (1.2 g/L), $FeSO_4{\cdot}7H_2O$ (48 mg/L), H_3BO_3 (11.6 mg/L), $CaCl_2{\cdot}2H_2O$ (10 mg/L), $MnCl_2{\cdot}4H_2O$ (7.2 g/L), $ZnSO_4{\cdot}7H_2O$ (0.88 mg/L), $CuSO_4{\cdot}5H_2O$ (0.32 mg/L), MoO_3 (72 μg/L), thiamine hydrochloride (40 μg/L), glucose (40 g/L), and glycine (0.5 g/L). All reagents were obtained from Sigma–Aldrich Co. and culture media were autoclaved at 121 °C for 15 min.

2.2. Photobioreactor system and conditions

C. protothecoides were cultivated in a photobioreactor system (Sartorious BIOSTAT PBR 2S, working volume 3L) with microalgae previously cultured in a flask as inoculum. The start-up medium had the same composition as the culture media, except for the glucose and glycine concentrations. Experiments were performed with two sets of initial media compositions varying the concentrations of glucose and glycine. One was used in modeling with the initial conditions of glycine and glucose as 0.5 g/L and 40 g/L, respectively. The other was used for validation of the model with 1 g/L of glycine and 20 g/L of glucose.

For the operation of the photobioreactor, two feed flow rates and the light intensity were manipulated at a predefined time calculated from D-optimal input design. The feed flow rate 1, f_1^i, is the nitrogen source that contains only glycine of 10 g/L. The feed flow rate 2, f_2^i, is for the carbon source supply that contains the same glucose and minerals with culture media, except KH_2PO_4 and K_2HPO_4. The photobioreactor was aerated at a rate of 50 mL/min, which contains 10 % of CO_2 by volume, and cultivated at 25 °C for 12 days. During the cultivation, 40 mL of microalgae was sampled every 12 h for data analysis.

2.3. Method for data analysis

Biomass concentration was determined by dry weight calculation of microalgae. 15 mL of sampled microalgae was centrifuged at 4000 rpm for 20 min. Centrifugation sediments were washed twice with distilled water and recentrifuged. The final precipitate were dried at 80 °C oven for 24 h and weighed.

The clear supernatant from centrifugation of sampled micralgae was filtered using a 0.22 μm syringe filter to remove any residuals in the liquid. After that glucose and glycine concentrations in the filtered supernatant were measured using HPLC (High Pressure Liquid Chromatography, Agilent 1260 Infinity). For the measure of glucose concentration, Zorbax carbohydrate column (4.6 mm ID, length 150 mm) at 30°C and a refractive index detector (RID) at 35°C were used. Acetonitrile and distilled water were mixed at a ratio of 75:25 and used as an eluent with a flowrate of 1.4 mL/min as mentioned in the user manual of Agilent zorbax carbohydrate analysis column.

The glycine concentration was also measured using HPLC with Zorbax Eclipse AAA column (4.6 mm ID, lenth 150 mm) and a variable wavelength detector (VWD). For the determination of glycine, 40 mM of Na_2HPO_4 solvent and a mixture of acetonitrile, methanol, and distilled water at a ratio of 45:45:10 were used as eluents. For the fluorescence detection of glycine, the automated OPA (Ortho Phthalaldehyde) derivatization method was applied (Henderson et al., 2000).

The total lipid concentration in the cells was determined using fluorespectrometer (Chen et al., 2009). For the detection of fluorescence, microalgae samples were stained with nile red solution. Nile red stains intracellular lipid droplets red and intensely fluoresce in a lipid rich environment. In this method, fluorescence intensity has a linear relationship with the lipid concentration. 0.15 mL of 10 μg/L nile red solution in ethanol and 2.7 mL of 30% (v/v) ethanol solution in water were added to 0.15 mL of each sample of microalgae. Samples were incubated at 40 °C for 10 min, and analyzed using fluorespectrometer. Excitation and emission wavelengths were selected as 530 nm and 604 nm, respectively. The fluoresence intensity was calibrated using microalgae samples, whose lipid concentration had been previously determined gravimetrically. For the extraction of lipid, hexane and isopropanol were used as solvents (Halim et al., 2011, 2012).

3. Model development

3.1. Development of photobioreactor model

The photobioreactor system used in this study manipulates nitrogen source feed (glycine), carbon source feed (glucose), and light intensity as inputs and analyzes the biomass, glycine, glucose, and lipid concentrations in the media as outputs. To predict the input–output relationship over the time course, a dynamic model