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Analysis and model delineation of marine microalgae growth and lipid accumulation in flat-plate photobioreactor

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ABSTRACT

A quantitative description of its growth and the "trade-off" between biomass production and lipid accumulation will provide a kinetic insight of the dependency of biomass and components on growth conditions and the interplays between lipid/protein production and N-limitation. The examination of the dependency of *Isochrysis galbana* growth on initial concentration of sodium nitrate ($NaNO_3$) in a flat-plate photobioreactor (FPPBR) revealed that the maximal lipid 106 mg L^{-1} is produced at 25 mg L^{-1} $NaNO_3$ and whereas the accumulation of biomass, protein and starch increases as $NaNO_3$ increases to 100 mg L^{-1}. The analysis and model fitting results indicate that *I. galbana* growth and production of lipids in a FPPBR can be quantitatively described by Baranyi-Roberts & logistic equation, and Luedeking-Piret model in a satisfactory manner, respectively. 25–75 mg L^{-1} was found to be proper level of $NaNO_3$ to achieve a good trade-off balance to produce both high quantities of biomass and lipids. Based on the predictions of growth model, after culturing middle stationary phase under the high nitrogen medium, switching of culture for algal cells to a nitrogen-free medium and/or high illumination intensity might be preferable strategy to achieve high level accumulation of lipids.

1. Introduction

Marine microalgae have been extensively used as the important baits of rotifers, bivalves and fish larva in the aquaculture industry, as the promising ingredients in food [1,2] and as the potential feedstock of biodiesel, due to their fast growth rate, high content of lipids, various beneficial components. In recent years, one of the focuses in algal research is the production of *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs); because the *n*-3 PUFAs, particularly EPA and DHA, can prevent and/or reduce the risk of some diseases, such as cardiovascular disease, cancer, inflammation and mental illness, etc [3–5]. The findings of Nuño et al. [6] indicated that supplement of *Isochrysis galbana* could help to maintain body weight and decrease the levels of glucose and cholesterol of diabetic animals. Therefore, marine microalgae with high levels of *n*-3 PUFAs have been a hot spot of the researches in aquaculture, food and pharmaceutical industries.

The biomass and chemical composition of microalgae are greatly affected by the environmental factors, such as light intensity, levels of nitrogen and carbon nutrients, salinity, temperature, and so on. In general, the growth rate and biomass yield of microalgae are largely dependent on the nitrogen supply in culture nutrients. Most of oleaginous microalgae show a higher growth rate and a lower production of lipid under nitrogen-sufficient conditions [7]. Instead, nitrogen-depletion or nitrogen-starvation presents enhanced lipid accumulation in microalgae, likely due to the flow of metabolic carbon from formation of carbohydrate and/or protein to lipid [8,9]. Thus, for optimizing production of lipid and protein etc bioproducts, it is very important to gain insight into the trade-off relationship among microalgae biomass, lipid and nitrogen-level in a system during the cultivation period.

Kinetic modeling is regarded as a useful tool to effectively understand metabolic processes, to optimize process parameters, and to evaluate cell growth, product formation and substrate consumption, in the scaling-up of bioreactors. The kinetic model derived from experimental data by means of mathematical tool in general is capable to accurately predict the growth behavior of microal-

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gae; and offers a reliable base to model scaling-up operation. Yang et al. [10] developed a mathematical model to describe *Chlorella minutissima* UTEX2341 growth, lipid production and glycerin consumption in a 2L batch bioreactor. Kumar and Das [11] used the logistic equation to develop the modeling and simulation of the growth profile of bubble column and airlift reactor and found that airlift reactor yielded a better growth profile and performance and a lower volumetric mass transfer coefficient K_{La} value than bubble column.

Flat-plate photobioreactors (FPPBRs) are widely used to cultivate different kinds of microalgae [12–15]. In order to improve the biomass and bioproduct yields, many FPPBRs are designed and/or configured to improve the nutrient distribution, increase the transferability of gas-liquid and fluid dynamics, and enhance the availability of light. Huang et al. [16] designed a FPPBR with novel mixer demonstrated that biomass concentration of algae can be significantly improved, by increasing the radial velocity of fluid and programing light/dark cycles of the FPPBRs. The biomass productivity of *Chlorella vulgaris* 31 in a novel FPPBR equipped with horizontal baffles was 1.88 times higher than that of FPPBRs without baffles [17]. Feng et al. [14] presented high lipid content, lipid concentration, and lipid productivity of *Chlorella zofingiensis* in 60 L FPPBRs outdoors up to 54.5%, 536 mg L^{-1} and 22.3 mg L^{-1} day^{-1}, respectively. However, there have been very few studies concerning kinetic models of algal growth and lipid production in a FPPBR.

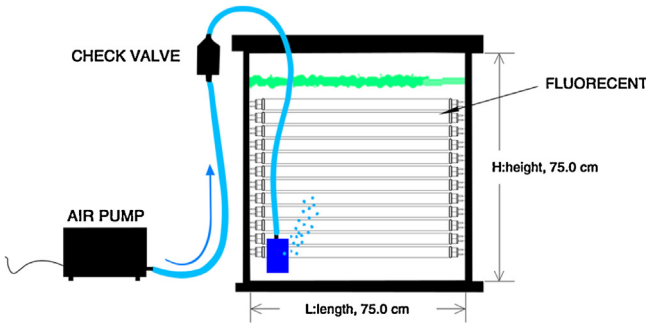
This study thus aims to construct a kinetic model to delineate the growth behaviors of *I. galbana* under different concentrations of sodium nitrate in a FPPBR (Scheme 1). Kinetic models covering algae growth, lipid formation and sodium nitrate consumption were used to determine the optimum parameters and explain the behavior of cultivating *I. galbana* in the FPPBR. Furthermore, the effects of important parameters in the kinetic models on the model performance were also addressed. The kinetic model derived from the experimental observations; which represents a quantitative characterization of the correlations between biomass and components with growth conditions, as well as a true reflection of "trade-off" between biomass production and lipid accumulation, is thus believed to be capable to perform a reliable prediction for a scale-up FPPBR or may also be beneficial for design of other reaction systems for microalgae cultivation.

2. Materials and methods

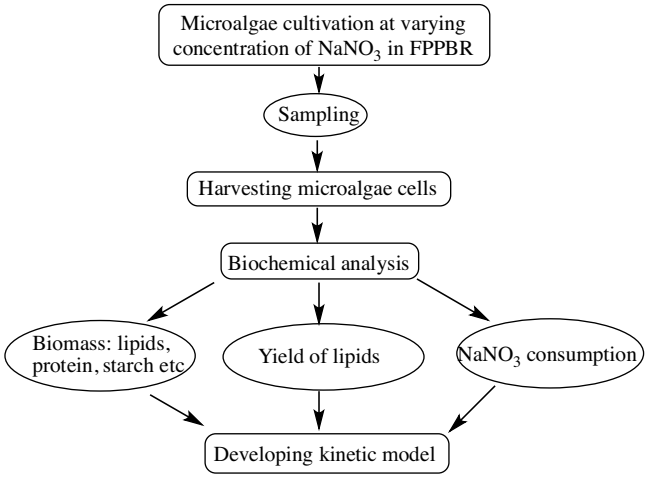
2.1. Microalgae and medium

A strain of *I. galbana*, donated by Prof. Qiu-jin Zhang, College of Life Science, Fujian Normal University, was used for this study. The microalga was cultured under the autoclaved seawater enriched with f/2 medium in a 25L simple flat-plate photobioreactors (FPPBR). All nutrient solutions and sea water were autoclaved at 121 °C for 30 min. The vitamin solution B_1 and B_{12} were filtered by 0.22 μm filter. The medium were composed of (mg L^{-1}): $NaNO_3$, 75; $NaH_2PO_4{\cdot}H_2O$, 6; Na_2EDTA, 4.36; $FeCl_3{\cdot}6H_2O$, 3.16; $CuSO_4{\cdot}5H_2O$, 0.01; $ZnSO_4{\cdot}7H_2O$, 0.025; $CoCl_2{\cdot}6H_2O$, 0.012; $MnCl_2{\cdot}4H_2O$,0.18; $Na_2MoO_4{\cdot}2H_2O$ 0.007; Vitamin B1, 0.1; Vitamin B12, 1.0×10^{-3}; Vitamin H, 1.0×10^{-3}. The pH and salinity of culture were set to 7.8 and 28 gL^{-1}, respectively. The initial density of cells was adjusted to 1.0×10^6 cells mL^{-1}.

This work involves cultivation, sampling, analysis and kinetic model development. A typical work flow chart is presented in Scheme 2.



Scheme 1. Schematic diagram of the flat-plate photobioreactor. L: length, 75.0 cm; H: height, 75.0 cm; W, width, 4.5 cm.



Scheme 2. Representation of work flow chart: cultivation, sampling, analysis and model development.

2.2. Flat-plate photobioreactor (FPPBR)

A 25L FPPBR (Scheme 1) equipped with an air filter and twelve 25 W fluorescents was used for this study. Light intensity was set at 4000 Lux over a 16:8 h light/dark cycle. The clean air bubbling velocity of FPPBR was controlled at 20L min^{-1}.

2.3. Analytical methods

2.3.1. Biomass measurement

100 mL culture was centrifuged at 5000 rpm for 10 min, washed twice with distilled water and dried in an oven at 80 °C to a constant weight. Optical density was measured by a UV spectrophotometer (UV-9600, Beijing, China) at 680 nm using fresh sea water as a blank. The relationship between dry biomass weight (X, mg L^{-1}) and OD_{680} for biomass estimation [20] (the correlation coefficient $R^2 = 0.996$) is:

$$X = 144.24 \times OD_{680} + 1.61$$

2.3.2. Chlorophyll measurement

1 mL culture was centrifuged at 10,000 rpm for 2 min and washed twice with distilled water. The cell sediment was fully mixed with 1 mL methanol in a vortex mixer for 5 min. After centrifugation, the absorbance of supernatants was determined at 652 nm and 665 nm using a UV spectrophotometer (UV-9600, Bei-

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