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Full-scale validation of an algal productivity model including nitrogen limitation

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ABSTRACT

Accurate predictions of algal productivity under nutrient-limiting conditions are needed to assess the economics of full-scale algal cultivation for the developing markets of food, feed, and at longer term, green chemistry and biofuel. In particular, predicting pigments production from micro-algae is a critical milestone in the assessments of high-value chemicals production from micro-algae. This study validates a mathematical model predicting algal biomass productivity in outdoor raceway ponds under nitrogen-limiting conditions. The model was first validated from experimental data collected during *Dunaliella salina* cultivation in indoor photobioreactors and accounts for the impact of light, temperature, and nitrogen concentration on algal productivity (overall accuracy on algal concentration of ± 2.7 mg L^{-1}, $N = 48$). The model was then validated against data collected in outdoor raceway ponds over a period of 2 years, representing a total of 111 days of cultivation. Biomass and extracellular nitrogen concentrations predictions were accurate within ± 0.055 g L^{-1} ($N = 69$) and ± 0.0024 g L^{-1} ($N = 26$), respectively. Model inaccuracies were mostly due to measurement errors and uncertainties on model inputs. Measured carotenoids concentrations were found proportional to the biomass concentrations in the outdoor raceway ponds. By coupling this linear correlation to the productivity model, predicted carotenoids concentrations were in good agreement with experimental data (accuracy within ± 0.0046 g L^{-1}, $N = 55$). The mathematical model developed in this study has therefore the potential to refine previous assessments of algal cultivation for biofuels and pigments production.

1. Introduction

Assessing the economics of full-scale algal production has been the object of numerous studies in the last years to estimate profitability of biofuel, food and high-value compounds production [28,32]. A key factor in these assessments is the algal productivity that can be reached at full-scale, as revenues per hectare facility are approximately proportional to the areal productivity. Accurate predictions of algal productivity in outdoor cultivation systems are therefore needed to assess the economics of algal cultivation. Within this context, various mathematical models were developed to predict algal productivity (see 5,10,25] for model reviews) and some of these models were even validated against outdoor data representing full-scale conditions (e.g. [4,14,30].). However, to the best of our knowledge, no model accounting for nitrogen limitation was validated against full-scale data. This particularly limits the applicability of productivity models for biofuel production, as lipid production is well known to be triggered by nitrogen starvation [23]. As a result, it is today difficult to estimate algal productivity in cultivation systems for biofuel production. Another application of algal cultivation is the production of high-value chemicals such as pigments [32]. Yet, to the best of our knowledge, no model of pigment productivity was validated against full-scale data.

Within this context, the objective of this study was to propose a fast approach to predict outdoor biomass and beta-carotene productivity from lab experiments. The proposed mathematical model of algal productivity accounts for nitrogen limitation. The model was first validated against *Dunaliella salina* cultivation data in indoor photobioreactors. Model predictions were then compared against data collected in outdoor raceway ponds dedicated to beta-carotene production.

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2. Materials and methods

2.1. Cultivation conditions in an indoor reactor

The data collected by Bonnefond et al. [11] during the indoor cultivation of *D. salina* (CCAP 18/19) was used to validate the model developed in this study. *D. salina* was indeed cultivated under fluctuating light and temperature conditions as described by Bonnefond et al. [11]. The indoor reactors were operated as fed-batch systems (dilution rate D of 0.69 and 0.70 d^{-1} in the two experiments) and the nitrogen concentration in the inflow was 0.4 mmol L^{-1}. The fixed carbon concentration (in g CL^{-1}), the nitrogen concentration in the medium and the nitrogen intra-cellular quota were continuously measured during the cultivation. The dry weight concentration (in g DW L^{-1}) in the reactors was calculated from the fixed carbon concentration based on a carbon content of 51% (calculated as the average C contents measured in the studies of Becker [7], Ben-Amotz et al. [8] and Renaud et al. [31]).

A mass balance on the nitrogen revealed that between 21% (experiment 1) and 27% (experiment 2) of the nitrogen introduced in the reactor during the entire cultivation period was "lost", i.e. not accumulated in the system or exiting the system. This can be explained by different practical reasons such as inaccurate nitrogen concentrations/quotas measurements or simply biological contamination. To compensate for this loss, the nitrogen concentration in the inflow was assumed to be 21% and 27% lower than the theoretical concentration of 0.4 mmol L^{-1}.

2.2. Cultivation conditions in outdoor raceway ponds

The algal species *Dunaliella salina* (MCCV 021) was cultivated in the raceway ponds operated over the duration of the Salinalgue Research Program (2010–2014) at Gruissan in France (Latitude: 43.094 N; Longitude: 3.084 E). This strain was preliminarily isolated from nearby salt ponds, for its ability to accumulate large amounts of beta-carotenes, even in conditions of moderate nitrogen limitation. The facility was composed of 4 raceway ponds (surface area: 250 m^2, depth: 0.5 m) and several closed photobioreactors used for culture inoculation. Six different batch cultivations were performed between 2013 and 2014, representing a total of 111 days of batch cultivation (Table 1). Ponds were initially filled with 50 m^3 of Conway medium [17] and inoculated with *D. salina* cultures preliminary grown in 10,500-L plastic bags (initial algal concentration in the raceway ponds was around 0.02 g L^{-1}). A paddle wheel ensured that ponds were completely mixed. pH was controlled at 6.5 by CO_2 injection to ensure both optimal pH conditions and also non-limiting CO_2 supply. Nitrate was also added into the culture during Batch 2 to avoid nitrate limitation. Because of evaporation at the pond surface, culture volume decreased over time and water was punctually added into the pond to compensate for evaporation losses.

Table 1
Summary of cultivation conditions for each batch study.

Batch number	Start date - End date	Nitrate addition date	Number of measurements				
			Temp.	Water level	OD	N. conc.	Caro. conc.
1	9 Sep.–7 Oct. 2013	NA	1395	19	20	6	6
2	4 Jun.–8 Jul. 2014	24 Jun 2014	1354	24	26	17	21
3	25 Jul.–8 Aug. 2014	NA	0	11	11	1	10
4	6 Aug.–21 Aug. 2014	NA	754	11	11	9	10
5	21 Aug.–3 Sep. 2014	NA	0	13	11	0	10
6	27 Aug.–4 Sep. 2014	NA	374	6	6	2	5
Total			3877	84	85	35	62

2.3. Measurements during outdoor cultivation

Various measurements were taken during outdoor cultivation of *D. salina*:

- Pond Temperature: Temperature was continuously monitored in the raceway pond during Batches 1, 2, 3, and 6 (See Table 1 for details)
- Meteorological data: Solar irradiance (accuracy: ± 5%), air temperature (accuracy: ± 0.3 °C), wind velocity (accuracy: ± 1 m s^{-1} or ± 5%, whichever is the greater), relative humidity (accuracy: ± 2%) and rain rate (accuracy: ± 5% for rates lower than 127 mm h^{-1}), were continuously measured during batch experiments (Vantage Pro2, Davis instruments Weather station, located within 100 m from the raceways)
- Pond salinity: Culture salinity was measured daily by refractrometry (accuracy: ± 2 g NaCl L^{-1}; Hand refractometer with Automatic Temperature Compensation, *Euromex*, Holland). As the times at which water was added into the pond to compensate for evaporation were not recorded, significant salinity variations were used to detect water additions into the pond (see S1 for further details).
- Water level: Water level was regularly measured using a ruler.
- Optical density: Culture optical density was measured daily at 680 and 800 nm (accuracy: ± 0.003 for absorbance value lower than 0.6 and ± 5% for absorbance values between 0.6 and 2; Spectroquant Pharo 100, Merck spectrophotometer). Optical density measurements were used to determine the biomass concentration by using the correlation presented in Section 2.3.
- Nitrate concentration: Nitrate concentration was measured daily by photometry (accuracy: ± 0.4 mg N L^{-1}; Spectroquant® nitrate test kits).
- Carotenoids concentration: Carotenoids concentration (mainly beta-carotene) was measured daily by using the technique described by Lichtenthaler [26].

2.4. Calibration between optical density and dry weight concentration

Biomass concentration was determined from optical density measured at 680 nm by using the following empirical correlation:

$$X = 0.491\ OD_{680} \quad (1)$$

where X is the biomass concentration (g L^{-1}), and OD_{680} is the optical density at 680 nm. This correlation originates from 32 measurements of both optical density and dry weight concentration of outdoor cultures of *D. salina* in raceway ponds under greenhouses located at Villefranche-sur-Mer (R^2 = 0.358, see S2 for details). The protocol described by Zhu and Lee [34] was used to determine sample dry weight concentration. Estimating dry weight concentrations from optical density measurements at 680 nm may be inaccurate due to change in pigment contents in cells and it is usually recommended to use optical densities for higher wavelengths as for example performed by Cornet et al. [18], Dermoun et al. [20], or Kurano and Miyachi [24]. However, the optical densities at 680 nm and 800 nm measured in the Salinalgue raceways were shown to be linearly correlated (R^2 = 0.984, N = 77; data not shown), suggesting that biomass concentration should be proportional to optical density at 680 nm.

2.5. The model

The model used in this study is the result of multiple modeling studies carried by our research group and couples several sub-models to predict the evolution of biomass concentration in the outdoor raceway