Opportunities to improve the areal oil productivity of microalgae

Guido Breuer [a,*], Packo P. Lamers [a], Marcel Janssen [a], René H. Wijffels [a,b], Dirk E. Martens [a]

[a] Bioprocess Engineering & AlgaePARC, Wageningen University and Research Centre, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
[b] Faculty Biosciences and Aquaculture, University of Nordland, N-8049 Bodø, Norway

HIGHLIGHTS

- Growth and TAG production in nitrogen-starved *Scenedesmus obliquus* is modeled.
- The potential for improvement of various technologies is evaluated using the model.
- The areal TAG productivity could be improved by up to 5-fold.
- TAG productivities up to 10.9 g m^{-2} day^{-1} seem feasible with near-term technology.

ARTICLE INFO

ABSTRACT

Microalgae are often considered as a promising alternative source of vegetable oils. These oils can be used for food and biofuel applications. Productivities that are projected for large-scale microalgal oil production are, however, often poorly supported by scientific evidence and based on too optimistic assumptions. To facilitate the inclusion of the microalgal physiology in these projections, existing knowledge and novel scientific insights were condensed into a mechanistic model that describes photosynthesis and carbon partitioning during nitrogen starvation. The model is validated using experimental data from both wild-type and a starchless mutant of *Scenedesmus obliquus*. The model is subsequently used to quantify how reactor design, process design, and strain improvement can improve the oil productivity from 2.1 to up to 10.9 g m^{-2} day^{-1}. These projected productivities are used to reflect on commonly assumed oil productivities and it is concluded that the microalgal oil productivity is often overestimated several folds.

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1. Introduction

Global demands for food and biofuels increase rapidly, together with an increase of concerns for depleting fossil resources and climate change (Draaisma et al., 2013). New sustainable sources of vegetable oil are therefore highly desired. Arable land to produce these vegetable oils is however limited (Godfray et al., 2010). Microalgae have the potential to achieve much higher oil productivities than commonly used terrestrial plants and can be cultivated on non-arable land (Wijffels and Barbosa, 2010). Microalgae-derived oils can be used as an edible oil (Draaisma et al., 2013; Klok et al., 2014), and can be used for the production of fatty-acid-methyl-esters, that can be used as a diesel–fuel substitute (Chisti, 2007). Microalgae are therefore often considered as a promising alternative natural-source of vegetable oils (this oil is from here on referred to as triacylglycerol; TAG) (Draaisma et al., 2013).

Although microalgae can potentially achieve much higher areal productivities than terrestrial plants, uncertainty remains about how high this productivity can be. Commonly used projections seem to overestimate this productivity as they are often poorly supported by scientific evidence and based on too optimistic assumptions (Moody et al., 2014; Waltz, 2009).

For example, often maximum photosynthetic efficiencies are combined with maximum TAG contents. These maxima do however not coincide. High photosynthetic efficiencies are only achieved under optimal growth conditions and low light intensities. Maximum TAG contents are only achieved during prolonged nutrient starvation, of which nitrogen starvation is most commonly used (Breuer et al., 2012). The microalgal response to nitrogen starvation is complex, and affects both photosynthesis and carbon partitioning. During nitrogen starvation, photosynthesis and carbon assimilation continue for a certain period, but the photosynthetic efficiency progressively decreases (Breuer et al., 2012; Simionato et al., 2013). TAG and starch are the main products made during nitrogen starvation but these are produced in a continuously changing ratio (Breuer et al., 2014).

* Corresponding author. Tel.: +31 317 485012.
  E-mail address: guido.breuer@wur.nl (G. Breuer).

Because this physiological response to nitrogen starvation is not taken into account in most productivity projections, a large uncertainty is introduced in techno-economic and life-cycle assessment studies of microalgal technology. Projections that are supported by a detailed quantitative understanding of the microalgal physiology during nitrogen starvation are therefore highly desired and can improve the quality of these projections.

Models, that closely follow the mechanisms behind photosynthesis and carbon partitioning, are excellent tools to provide the basis for these productivity projections, can be used for an evaluation of technology options, and can be used to investigate the potential of improvement strategies. For example, a model can help to quantify how biological traits, process conditions, and process design can be exploited to enhance TAG production.

Models that describe a detailed and mechanistic response of both photosynthesis and carbon partitioning during nitrogen starvation were previously lacking. Existing models focus either on photosynthesis (Geider et al., 1998; Packer et al., 2011; Quinn et al., 2011) or carbon partitioning (Dillschneider and Posten, 2013; Klok et al., 2013; Mairet et al., 2011) during nitrogen starvation and often use an over-simplified or empirical approach to model photosynthesis or carbon partitioning. Therefore, in this work a novel mechanistic model is developed, using *Scenedesmus obliquus* as a case-study, that is the first to independently describe the effects of nitrogen starvation on photosynthesis and carbon partitioning, including inter-conversion of biomass constituents and considers photosynthetic and inter-conversion yields specific for each biomass constituent. This model is developed to describe the behavior of both wild-type and the slm1 starchless mutant of *S. obliquus* (Breuer et al., 2014; de Jaeger et al., 2014), but could easily be adapted to describe the behavior of other microalgae species. The model is validated using nitrogen run-out cultivations of wild-type and the slm1 starchless mutant of S. *obliquus* using the experimental data presented by (Breuer et al., 2014).

Subsequently, the model is used to quantify how improvements in the carbon-partitioning mechanism, photosynthesis, process design, and reactor design can improve the TAG productivity. These findings are finally used to provide productivity projections for current and future large-scale microalgal TAG production.

2. Methods

2.1. Experimental data

In this work, the experimental data as presented by (Breuer et al., 2014) is used. Nitrogen run-out batch cultivations were performed for wild-type *S. obliquus* UTEX 393 (wt) and the slm1 starchless mutant of *S. obliquus* (de Jaeger et al., 2014). Experiments were performed in a 1.7 l flat-panel airlift-loop photobioreactor (Labfors 5 Lux, Infors HT, Switzerland) that was aerated with air enriched with 2% CO_2 at 1 l/min. In these cultivations, the algae were inoculated at a low biomass density (less than 0.05 g/l) and cultivated at an incident light intensity of 100 μmol m^{-2} s^{-1} until the biomass density reached 0.3–1 g/l after which the incident light intensity was increased to 500 μmol m^{-2} s^{-1}. The reactor was continuously illuminated (24 h/day) at kept at 27.5 °C and pH 7. KNO_3 was the limiting nutrient (initial concentration of 10 mM) and was depleted at a biomass concentration of 1.5 g/l. Periodically a sample was taken and analyzed for biomass dry weight, total fatty acid (TFA), TAG, starch, and dissolved NO_3^- concentration, and absorption cross section as described by Breuer et al. (2014). These experiments were performed in biological duplicates and these duplicate datasets were merged into one dataset for parameter estimation as explained in Supplementary data 1.6.

2.2. Parameter estimation

The incident light intensity, light path of the reactor, absorption cross section of nitrogen replete biomass and nitrogen content of nitrogen replete biomass were directly derived from the experimental conditions or experimental observations, and were used as constants in the model (Supplementary Table 1). Parameters related to partitioning of light energy between TAG and starch synthesis and related to degradation of starch were only relevant for the wt. This results in 8 model parameters to be estimated for the wt and 4 model parameters to be estimated for the slm1 starchless mutant. Initial estimates for these parameters were made from the experimental data, as described in Supplementary data 1.6. Subsequently, parameter estimates were improved by minimizing the normalized sum of squared differences (ssq) between model predictions and experimentally observed dry weight concentration and starch and TAG contents throughout the cultivation (Supplementary data 1.6).

Because local minima for ssq were observed, a Monte-Carlo algorithm was used in which ssq was calculated for combinations of parameters that were randomly distributed around the aforementioned initially estimated values. The combinations of model-parameter values that resulted in the lowest ssq-values were manually evaluated and it was confirmed that the trends of the model were consistent with the trends as observed in the experimental data (as described in more detail in Supplementary data 1.6). Finally, the combination of parameters that best fit the experimental data was further optimized using unconstrained non-linear optimization (Matlab: fminsearch) to yield the values as presented in Supplementary Table 1.

2.3. Flux balance analysis

In this work, the metabolic network for *Chlamydomonas reinhardtii* as described by (Kliphuis et al., 2012) is used with some modifications as described in detail in Supplementary data 1.3. The reversibility of reactions is chosen according to (Kliphuis et al., 2012). The number of reactions in the network is largely reduced by lumping sequential non-branched reactions into single reactions. This results in 48 metabolites and 59 reactions. See Supplementary Fig. 3 and data 2 for an overview of all reactions and their reversibility.

To calculate the theoretical maximum photosynthetic yields of each biomass constituent (M), flux balance analysis is used to find a flux distribution that result in the highest possible production rate of each of these biomass constituents, at a fixed supply rate of photons, while the stoichiometry and reversibility of the reactions are constrained (as described in more detail in Supplementary data 1.3).

The photosynthetic yield of product M can then be calculated as the ratio between the production rate of product M and the photon supply rate. To calculate the interconversion yield of starch to TAG, the production rate of starch (v_{starch}) is set to -1, the photon supply rate is set to 0, and the TAG production rate (v_{TAG}) is maximized. The yield is calculated as the ratio between $v_{TAG}/-v_{starch}$.

2.4. Impact of model parameters on the photosynthetic TAG yield

Using the presented model, the impact of various model parameters, that represent, or can be influenced by, the reactor design, process design, carbon-partitioning mechanism, or photosynthetic machinery were investigated. To do so, model simulations were performed using Monte-Carlo-sampled combinations of the incident light intensity (I_0), biomass dry weight concentration at the onset of nitrogen starvation ($c_{DW,n=0}$), and reactor thickness (z), and for combinations of the incident light intensity, the pigmentation at