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Modeling the competition between antenna size mutant and wild type microalgae in outdoor mass culture

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ABSTRACT

Under high light conditions, microalgae are oversaturated with light which significantly reduces the light use efficiency. Microalgae with a reduced pigment content, antenna size mutants, have been proposed as a potential solution to increase the light use efficiency. The goal of this study was to investigate the competition between antenna size mutants and wild type microalgae in mass cultures. Using a kinetic model and literature-derived experimental data from wild type *Chlorella sorokiniana*, the productivity and competition of wild type cells and antenna size mutants were simulated. Cultivation was simulated in an outdoor microalgal raceway pond production system which was assumed to be limited by light only. Light conditions were based on a Mediterranean location (Tunisia) and a more temperate location (the Netherlands). Several wild type contamination levels were simulated in each mutant culture separately to predict the effect on the productivity over the cultivation time of a hypothetical summer season of 100 days. The simulations demonstrate a good potential of antenna size reduction to increase the biomass productivity of microalgal cultures. However, it was also found that after a contamination with wild type cells the mutant cultures will be rapidly overgrown resulting in productivity loss.

1. Introduction

One of the most important bottlenecks in microalgae cultivation is the inefficient utilization of light energy under high irradiance. Microalgae are easily oversaturated with light which strongly reduces the light use efficiency. This limitation can be alleviated by genetically reducing the amount of light energy that is absorbed per cell. The creation of such antenna size mutants, i.e., microalgae with a reduced pigment content, has been proposed as a potential solution (Formighieri et al., 2012; Kwon et al., 2013; Mussgnug et al., 2007a; Oey et al., 2013; Ort et al., 2011; Perrine et al., 2012) to light saturation. The lower pigmentation of the mutants allows increased biomass concentrations with the same light gradient in the photobioreactor (Melis et al., 1998; Mussgnug et al., 2007b). The lower biomass specific light absorption rate leads to a higher productivity of mutant cultures than that of wild type cultures under high light conditions (Mussgnug et al., 2007b). In practice, some mutants were indeed reported to demonstrate improved growth characteristics under specific light conditions (Mussgnug et al., 2007b; Cazzaniga et al., 2014; Mitra and Melis, 2008).

Clearly, antenna size mutant generation, especially through directed mutagenesis, is an immature technology (de Mooij et al., 2014). Currently there are no suitable antenna size mutant strains available that can be analyzed for their performance in competition experiments. A better understanding of the photosynthetic machinery and an advanced genetic toolbox are required to create better mutants. Still, it is interesting to study the potential of antenna size reduction for mass cultivation of microalgae using model simulations. Extrapolation of laboratory data utilizing a predictive model that is constructed on a solid theoretical foundation is an attractive alternative to performing outdoor cultivation experiments. Modeling can function as a great tool to investigate the improvement of mass culture productivity using antenna size mutants. In addition, the impact of competition for light between mutant and wild type strains can be predicted. Competition between the antenna size mutant and its own wild type is expected to result in productivity losses. The outcome of such a modeling approach results in more realistic expectations and can be used to determine genetic engineering targets to optimize mutant cultivation.

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The aim of this study was to estimate antenna size mutant productivity in photobioreactor mass cultures and quantify the competition between the antenna size mutant and its wild type. Knowledge on growth properties of separate cultures (Hobson, 1969; Huisman et al., 1999) is required to analyze mixed cultures and to estimate the competitiveness of the involved species under light limiting conditions. Many models describing biomass productivity of microalgae are available to date (Acién Fernández et al., 1998; Cornet and Dussap, 2009; Geider et al., 1997; Huesemann et al., 2016; Lee et al., 2014; Quinn et al., 2011; Takache et al., 2012). These models differ by their level of complexity, model strategy and need for parameterization. In our study the productivity was calculated with a microalgal growth model which is based on summing up local rates of photosynthesis calculated for every position in the photobioreactor. The presented model takes into account the light gradient within the microalgal culture and also the wavelength dependency of light absorption. The model is based on a previously validated growth model for *Chlorella sorokiniana* under continuous light (Blanken et al., 2016), that was obtained by combining models of Jassby and Platt (1976) and Pirt (1965) and the Lambert-Beer law. The model has been modified by adding a carbon partitioning mechanism and simulated outdoor light conditions to describe growth under day/night cycles. By extending the model, it serves well to answer new research questions regarding the competition of microalgae strains that have different light harvesting capacities. The light model was further adapted to represent an outdoor microalgal raceway pond production system taking into account solar elevation. The wild type properties were defined based on experimental data of *C. sorokiniana*. Antenna size mutants were considered to only differ from the wild type in their reduced absorption cross section to clearly identify the potential of reduced antenna sizes. Productivities of the mutant monocultures with different antenna size reductions (0–90%) were investigated. Several wild type contamination levels were separately simulated in each mutant culture to predict the effect on the productivity over the cultivation time (100 days).

2. Model description

2.1. Structure

With the kinetic model the biomass productivity of a microalgae mass culture is calculated as a function of the incident light intensity during the diurnal cycle. The change of the spectral composition with increasing reactor depth due to the preferential light absorption of microalgae was taken into account. The model allows for the calculation of biomass productivity of an antenna size mutant culture before, during, and after contamination with its wild type. In all of the cases, light is assumed to be the limiting factor for growth and, consequently, mutant and wild type are competing for light energy. An extensive description of the model can be found in Appendix A.

To account for the effects of the diurnal cycle and carbon partitioning, functional biomass and intracellular carbohydrate reserves were regarded as separate compounds. The carbohydrate reserves are referred to as 'sugar' in this study. Consequently, for each strain, two production rates were distinguished: the production of functional biomass (X') and of sugar (S).

Refer to Fig. 1 for an overview of the model structure. The overall microalgae production model consists of three modules: Location, time, and wavelength dependent light distribution (Module 1); specific light absorption, and photosynthetic sugar production (Module 2); partitioning of sugar towards biomass, and a sugar reserve pool (Module 3). The first module describes the location-specific light intensity and the light distribution in the culture during the day. These are based on solar elevations, Snell's law, Fresnel's equations, and Lambert-Beer's law, while neglecting the effect of light scattering. The second module describes the sugar production as a function of specific light absorption and, consequently, depends on the local light intensity, biomass concentration, and strain-specific characteristics. The third module represents the allocation of photosynthetically produced sugar to maintenance related processes, to the accumulation of carbohydrate reserves, and to the production of functional biomass. In addition, the partitioning of sugar is differentiated between the day and night periods during the diurnal cycle.

2.2. Strategy and assumptions

Two ordinary differential equations (Eqs. (A9) and (A10)) describe the change in production and respiration of the functional biomass and the sugar for each strain separately. The Matlab built-in solver for stiff differential equations (ode23s) based on the 2nd/3rd order Runge-Kutta method was used to numerically integrate the change in concentrations over the cultivation time. This results in the respective functional biomass and sugar concentrations as a function of time.

The simulated cultivation time was 100 days. We simulated both a Mediterranean location (Tunisia, Lat. 36.867°) with a high irradiance and clear sky and a temperate location (the Netherlands, Lat. 52.117°) with considerable cloud cover. One specific summer day (July 15th, day 196 of the year) was continuously repeated in the model for each location. All simulations began at 8:00 am local solar time. During the day, the dilution rate was maintained constant while there was no culture dilution during the night. The dilution rate was optimized for each strain and each location (Netherlands and Tunisia) to maximize the productivity (See Appendix D for optimization procedure). The optimal dilution rate was subsequently applied during the final simulations.

Theoretical antenna size mutants of *Chlorella sorokiniana* were simulated. The strain-specific characteristics $\left(\mu_m, m_S, Y_{X'/S}, q_S^{max}, x_{S,min}\right)$ of the mutants were kept identical to the experimentally determined characteristics of the wild type (Table A3 of Appendix E), i.e., only the biomass specific absorption cross section for each wavelength $\left(a_{X',\lambda}\right)$ was altered by multiplying $a_{X',\lambda}$ with 0.1-0.9, depending on the degree of antenna size reduction (90-10%). Throughout the entire day/night cycle, $a_{X',\lambda}$ was assumed to be constant. The wild type absorption cross section was measured under mass culture conditions with an incident light intensity of 1500 $\mu mol_{ph}\, m^{-2} s^{-1}$ and an outgoing light intensity of 10 $\mu mol_{ph}\, m^{-2} s^{-1}$. The antenna size mutation is assumed to be stable and not affecting any other cellular property, and the considered strains are assumed as not being sensitive to photoinhibition under the simulated light conditions. Different wild type contamination levels were considered as well as the scenario of a reverse mutation which was assumed to result in a contamination of one cell per cubic meter at the initiation of the 100-days simulation. One cell of *C. sorokiniana* corresponds to a mass of $1.4 \cdot 10^{-11}$ g, or 0.14 pg (Rosenberg et al., 2014). A reflective (80%) ground cover was simulated to be situated at the bottom of the photobioreactor pond to allow a fairer comparison of mutant and wild type performance.

The areal biomass productivity (r_{Cx}) was calculated by multiplying the total biomass concentration ($X' + S$) with the dilution rate (D) and the reactor depth (d_R):

$$r_{Cx} = (X' + S) \cdot D \cdot d_R \quad (1)$$