



Light attenuation in photobioreactors and algal pigmentation under different growth conditions – Model identification and complexity assessment

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ABSTRACT

Microalgae are photosynthetic organisms, and thus one of the most important factors affecting their growth is light. Yet, effective design and operation of algal cultivation systems still lacks robust numerical tools. Here, a comprehensive and mathematically consistent simulation model is presented in the ASM-A framework that can accurately predict light availability and its impact on microalgae growth in photobioreactors (PBR). Three cylindrical column reactors, mimicking typical open pond reactors, with different diameters were used to conduct experiments where the light distribution was monitored inside the reactor. A batch experiment was conducted where the effect of nutrients and light availability on the pigmentation of the microalgae and light distribution was monitored. The effect of reactor size and cultivation conditions on the light distribution in PBRs was evaluated. Moreover, we assessed the effect of using different simulation model structures on the model prediction accuracy and uncertainty propagation. Results obtained show that light scattering can have a significant effect on light distribution in reactors with narrow diameter (typical to panel-type PBRs) and under cultivation conditions that promote low pigmentation or low biomass concentration. The light attenuation coefficient was estimated using the Lambert-Beer equation and it was compared to Schuster's law. The light attenuation was found to be dependent on biomass concentration and microalgae pigmentation. Using a discretized layer model to describe the light distribution in PBRs resulted in the most accurate prediction of microalgal growth and lowest uncertainty on model predictions. Due to model complexity a trade-off needs to be made between accuracy of the prediction and simulation time.

1. Introduction

Optimizing microalgal cultivation is critical for effective reactor operation. One of the most important factors affecting microalgal growth is light availability [1]. Light is essential for microalgae to conduct photosynthesis and photoautotrophic cultivation is not viable without sufficient light in the reactor [2]. During photosynthesis microalgae convert carbon dioxide and water into carbohydrates and oxygen using light as an energy source [3]. In the light reactions, the light harvesting antenna collects the incoming light (i.e., photons) that is transported to the reaction centres (PSI and PSII) where this energy is converted into chemical energy in the form of NADPH₂ and ATP [4]. In the dark reaction or Calvin cycle the produced chemical energy is used to reduce carbon dioxide to phosphoglycerate, which can be further

converted to, e.g., carbohydrates [4]. In closed photobioreactors (PBR), the light is more efficiently distributed as a result of optimal reactor designs, e.g., flat-panel [5]. However, in open pond cultivation systems, 90% of the incoming light intensity is absorbed in the first few centimetres of the culture, resulting in an inefficient distribution of photons [6]. Consequently, effective mixing is required to ensure that microalgal cells are regularly exposed to light [7]. Therefore for proper design of algal cultivation systems, the application of process models that accurately describe light distribution dynamics is essential [8]. Another factor affecting microalgae cultivation in open pond cultivation is the potential contamination by bacteria or protozoa [6]. Open cultivation of microalgae is used especially in used water resource recovery systems, where the potential for bacterial contamination is high [9]. The presence of bacteria can further affect the light distribution in PBRs.

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There are two major groups of photosynthetic pigments in green algae: chlorophylls (green pigment), absorbing in two spectrum bands (blue (450–475 nm) and red (630–675 nm)), and carotenoids (yellow pigment), absorbing at 400–550 nm. Chlorophylls are the main photon-harvesting pigments, whilst carotenoids can serve as protective pigments against high irradiance and reactive oxygen species and improve the light absorbance and the light utilization [10,11]. Depending on the culture conditions – mainly nitrogen and light availability – chlorophylls and carotenoids are expressed in different quantities [12–15]. Pigments are also important high value products that can be used as, e.g., food and feed ingredients or cosmetics [11,16–18].

Typically, there are three distinct light regimes prevailing through algal growth. Under light limited conditions, photosynthesis shows linear dependency on light intensity. The maximum photosynthetic rate is reached at saturation light intensity, from where the photosynthetic rate is limited by the dark reactions [2]. Light intensity that is higher than the saturation level causes photoinhibition, whereby the photosynthetic rate declines due to non-photochemical quenching to dissipate the excess energy as heat [19]. Algae exposed to inhibiting light intensities for more than 1 min will be affected by photoinhibition [19]. Due to light dynamics, microalgae have developed acclimation mechanisms to cope with light intensity changes. Regulation occurs in the reaction centres, mainly in PSII, by altering their photon-harvesting capacity or the number of reaction centres [20]. Under light limiting conditions microalgae increase the amount of chlorophyll, i.e. their photon-harvesting capacity. Under high light intensity, chlorophyll levels are reduced to avoid excess energy harvesting [19].

Light attenuation in the PBR is affected by the absorption capacity of photosynthetic pigments, the shading effect by cells and light scattering caused by reactor wall and cells [10]. The Lambert-Beer expression accounts for the light absorption in the reactor by the biomass concentration [21] or by the combination of biomass and pigments concentration [22], but does not account for scattering. Schuster's law can be used in cases where the light scattering is considered [21]. When the pigment concentration impact on light distribution is considered, it is necessary to include pigments concentration in the biological model as a state-variable. There are several approaches to model pigment concentration: i) relating the intracellular chlorophyll content to the internal nitrogen quota [22] or to the nitrogen assimilation [23], ii) considering photo-acclimation as the driving force of chlorophyll accumulation [20], or iii) relating the chlorophyll synthesis to inorganic carbon uptake [24]. The dependence of microalgal growth on light intensity can be modelled by following three complexity levels [19]. Type I consists of biokinetic models that employ incident or average light intensity, i.e., algal cells are assumed to be exposed to the same light intensity through the entire reactor volume and have the same photosynthetic rate, thus neglecting the effect of photo-acclimation and light attenuation (see, e.g. [25]). Type II models account for light distribution in the culture by applying, e.g., the Lambert-Beer expression (e.g. [21,26]), to predict the light intensity at a given reactor depth. Finally, type III models account for culture history in terms of light exposure as cells move around in the system (e.g. [27]). Light intensity is commonly measured and expressed in the photosynthetically active radiation (PAR) range (400–700 nm) (e.g. [5,28,29]).

A microalgal biokinetic process model developed in the framework of activated sludge modelling (ASM-A) was proposed earlier [25], including photoautotrophic and heterotrophic microalgal growth, nitrogen and phosphorus uptake and storage and biomass decay processes. The effect of light intensity on photoautotrophic growth was experimentally assessed and found to be best described by the Steele equation. An average light intensity is used to account for light intensity inside the reactor (i.e., Type I model). Moreover, in the paper, the effect of light intensity on heterotrophic growth was assessed. The goal of the ASM-A model is to move towards a consensus based process model for green microalgae. As discussed above, light intensity within PBRs can

be accounted for in different ways, which was not evaluated in the original ASM-A biokinetic process model. Thus, to further develop a comprehensive process modelling framework for green microalgae, in this paper, different approaches to predict the effects of light intensity on microalgal growth are assessed.

Hence, the objectives of this study are: (i) to assess the distribution of light intensity in column reactors used for microalgae cultivation with different dimensions, biomass concentrations and pigmentation, receiving light from the top; (ii) to assess the effect of cultivation conditions on the light distribution and the pigment synthesis during batch cultivation; (iii) to identify a process model structure that can describe pigments accumulation and degradation as a function of substrate availability; (iv) to compare different simulation model complexity levels used to predict light intensity in PBRs.

2. Materials and methods

2.1. Microalgae and culture media

A mixed green microalgal consortium consisting mainly of *Chlorella sorokiniana* and *Scenedesmus* sp. was used in this study [25]. The mixed culture was cultivated using the MWC + Se synthetic medium [30] by adjusting the nitrogen and phosphorus concentrations as later specified. The consortium was also grown in effluent water from a laboratory-scale enhanced biological phosphorus removal (EBPR) system [31] operated at 16 days of solids retention time (SRT) fed with pre-clarified used water from Lundtofte WWTP (Kgs. Lyngby, Denmark).

2.2. Microalgal cultivation in batch reactors

Batch experiments were carried out in an 8-L batch reactor (made out of clear acrylic material, see Fig. S1, Supporting Information (SI)), to assess the effect of nutrients and light availability on the pigments concentration of the microalgae. The cylindrical reactor had a diameter of 140 mm, height of 0.6 m and working volume of 8-L. Constant aeration with CO₂ enriched air (5% CO₂) at a flow rate of 20 L/h was used to mix the biomass and to provide CO₂. Light was supplied from the top of the reactor with a custom-built lamp, providing $1500 \pm 150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, with a metal-halide light bulb (OSRAM®, Germany). The reactor wall was covered with a black cloth from the outside to reduce the effect of ambient light on the monitoring of the incoming light intensity. The light sensor (described in Section 2.3) was only placed inside the reactor for the course of the light intensity measurements (otherwise it was kept outside of the reactor to not interfere with the light penetration). The inoculum for the batch cultivation was taken from a reactor where the culture was cultivated under light limited conditions due to high biomass concentration (data not reported). Moreover, the inoculum was grown in a modified MWC + Se medium, and kept under nutrients in excess conditions for the inoculation period (data not shown). The MWC + Se medium was modified to reach 7.55 mg NH₄⁺-N/L, 12.7 mg NO₃⁻-N/L and 3.5 mg PO₄-P/L. The reactor was kept at room temperature (23–24 °C). The pH of the algal culture varied in the range of 6.8–7.9 during the experiments. After 15 days of starvation, when nutrients were depleted in the cultivation medium, nitrogen and phosphorus were spiked again reaching 1.8 mg NH₄⁺-N/L, 6.6 mg NO₃⁻-N/L and 0.6 mg PO₄-P/L. Algae biomass was diluted by replacing 20% of the culture with fresh cultivation medium, thereby supplying other micronutrients that were likely depleted.

Moreover, three reactors (made out of clear acrylic material, Fig. S2, SI) of different diameters were used in the experiments where the effect of reactor size, nutrient availability and cultivation media on light attenuation were assessed. Reactor 1 had a diameter of 240 mm, height of 0.6 m and working volume of 22.5-L. Reactor 2 had a diameter of 140 mm, height of 0.6 m and working volume of 8-L. Finally, reactor 3 had a diameter of 110 mm, height of 1.2 m and working volume of 10.5-

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