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Light-field-characterization in a continuous hydrogen-producing photobioreactor by optical simulation and computational fluid dynamics

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Abstract

Externally illuminated photobioreactors (PBRs) are widely used in studies on the use of phototrophic microorganisms as sources of bioenergy and other photobiotechnology research. In this work, straightforward simulation techniques were used to describe effects of varying fluid flow conditions in a continuous hydrogen-producing PBR on the rate of photofermentative hydrogen production (rH_2) by *Rhodobacter sphaeroides* DSM 158. A ZEMAX optical ray tracing simulation was performed to quantify the illumination intensity reaching the interior of the cylindrical PBR vessel. 24.2 % of the emitted energy was lost through optical effects, or did not reach the PBR surface. In a dense culture of continuously producing bacteria during chemostatic cultivation, the illumination intensity became completely attenuated within the first centimeter of the PBR radius as described by an empirical three-parametric model implemented in Mathcad. The bacterial movement in chemostatic steady-state conditions was influenced by varying the fluid Reynolds number. The "Computational Fluid Dynamics" and "Particle Tracing" tools of COMSOL Multiphysics were used to visualize the fluid flow pattern and cellular trajectories through well-illuminated zones near the PBR periphery and dark zones in the center of the PBR. A moderate turbulence (Reynolds number = 12,600) and fluctuating illumination of 1.5 Hz were found to yield the highest continuous rH_2 by *R. sphaeroides* DSM 158 (170.5 mL L^{-1} h^{-1}).

Keywords

Photobioreactor, Optical ray tracing, Particle tracing, Computational Fluid Dynamics, Hydrogen

Introduction

Phototrophic microorganisms like algae, cyanobacteria and phototrophic bacteria have high potential to sustainably produce proteins, biofuels, cosmetics and pharmaceutical drugs (de Jesus Raposo et al., 2013; Singh et al., 2011). Especially, hydrogen has an undisputed future importance as a secondary source of energy and raw material (Jones and Mayfield, 2012). However, to meet growing demands for hydrogen, it is essential to develop sustainable processes for its bioproduction. Anaerobic phototrophs, such as the purple non-sulfur bacteria (PNS) *Rhodobacter sphaeroides* DSM 158, are promising candidates for the photobiotechnological production of hydrogen (Adessi and de Philippis, 2014; Weber et al., 2014). PNS bacteria belong to the metabolically versatile group of α- or β-proteobacteria and primarily grow photoheterotrophically under anaerobic conditions. Their hydrogen production is catalyzed by light-driven nitrogenase activity during nitrogen-limited cultivation conditions.

PBRs are classified as open or closed systems (Pulz, 2001). Open systems include natural waters, such as lakes or lagoons, and artificial ponds like tanks, circular or raceway ponds in which phototrophic microorganisms are exposed to natural solar radiation (sunlight). Pond systems are relatively cheap, easy to construct, and have low production and operating costs (Ugwu et al., 2008), but most open systems require large areas of land, and the cultivation conditions they provide are dependent on the climatic conditions of the location. Open systems are also highly susceptible to contamination and considerable evaporation losses. Consequently, closed PBR systems are preferable for process optimization, particularly at the laboratory scale, because critical factors (e.g. cell densities, the light regime, nutrient concentrations, partial gas pressures, temperatures, volumes and contamination) can be readily monitored and controlled.

Numerous lab-scale PBRs have been constructed with diverse geometries, mixing methods, and light sources (Wang et al., 2012). Widely used types of systems include flat panels (internally or externally illuminated), columns (airlift or bubble), and tubular or stirred tank reactors (Posten, 2009). Light energy for lab-scale PBRs is provided by various forms of artificial light sources, such as halogen lamps, fluorescent lamps, and light emitting diodes. Unlike organic substrates, light cannot be stored, pumped or mixed. Instead it has to be continuously supplied, typically from outside of the reactor, and ideally homogenously for each cell. To monitor and evaluate photobiotechnological processes it is essential to quantify the provided illumination intensity with great accuracy. However, to acquire reliable data for characterizing the cells' light environment several parameters must be considered including light path(s) from the source(s), its attenuation by self-shadowing of the cells and scattering within the cell suspension, and the trajectories of cells within the reactor induced by the mixing of the fluid phase. Hence, several recent studies have simulated the intensity of light emitted by artificial light sources used in various PBR systems, including: bubble columns with external or internal illumination (Bergeroglu et al., 2007) and reactor systems equipped with optical fibers (Csögör et al., 1999; Csögör et al., 2001; Xue et al., 2013), fluorescent lamps (Huang et al., 2012; Kong and Virgil, 2014) or light emitting diodes (Farges et al., 2009; Lee, 1999).

Since the histories of individual cells' light exposure within a PBR are also influenced by the fluid flow patterns, the combination of *Computational Fluid Dynamics* (CFD, which can simulate fluid flows induced by mixing) and optical simulation has high potential for improving process understanding (Nauha and Alopaeus, 2013, Perner-Nochta and Posten, 2007; Sato et al., 2012; Zhang, 2013). Generally, CFD refers to the formulation and solution of fundamental transport equations in a three-dimensional system. Recent research on the application of CFD for modeling and designing PBRs has primarily focused on optimal mixing in two-phased systems (gas-fluid) to ensure constant temperature, pH profiles and gas transfer conditions

(Bitog et al., 2011; Seo et al., 2012). However, in pioneering work Perner-Nochta and Posten (2007) demonstrated the potential value of combining CFD and optical simulations in an analysis of light/dark cycles for algal cells in a tubular PBR equipped with a static mixer.

Nevertheless, the description of light as the sole source of energy for photobiotechnological processes has often been neglected. Here, we introduce an approach for simulating optical ray traces to characterize in detail the intensity and distribution of illumination from the artificial light sources used to illuminate a lab-scale PBR. A three-parametric empirical model was used to simulate attenuation of illumination in a dense culture of continuously hydrogen-producing *R. sphaeroides* DSM 158 in a cylindrical PBR vessel. In conjunction with CFD analysis of the fluid flow pattern and particle tracing to define cellular trajectories in the PBR, the results provide detailed indications of the cell-specific temporal patterns of available light energy.

Materials and Methods

Medium and Inoculation

Rhodobacter sphaeroides DSM 158 was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). 50 mL of preculture medium (PCM, in a 100 mL air-tight-closed DURAN bottle), containing 3 g L^{-1} glutamic acid (Merck kGaA) and 0.3 g L^{-1} D/L lactic acid (Fluka) as nitrogen and carbon source, respectively, was inoculated with cryo-conserved bacterial stocks and incubated at 30 °C and 20 W m^{-2} halogen illumination in a thermostatically controlled water bath for 48 hours. Anaerobic conditions were achieved by flushing the liquid phase with helium for 10 minutes at a volumetric flow rate of 1 L min^{-1}. The preculture was sub-cultivated three times by transferring 5 mL of bacterial culture to 45 mL of fresh PCM and subsequent incubation for 48 hours at 20 W m^{-2} illumination intensity and anaerobic conditions before cells of the late exponential growth phase were used to inoculate the PBR containing the hydrogen production medium (HPM, 1.5 g L^{-1} glutamic acid (Merck kGaA)

and 3.6 g L^{-1} D/L lactic acid (Fluka)). The additional medium components of the HPM and reactor inoculation procedure were as previously reported by Krujatz et al. (2015).

Batch and chemostatic operation of the PBR

The PBR consisted of a cylindrical, transparent, Applikon 1 L stirred tank reactor equipped with an external illumination device and three six-bladed Rushton impellers for stirring, as illustrated in Figure 1. The halogen light sources the illumination device was fitted with provide light in the whole range of the photosynthetic active radiation nedded by PNS bacteria including radiation between 400 nm and 500 nm (carotenoids) and near-infrared radiation between 750 nm - 900 nm (bacteriochlorophyll a). A detailed description of the batch and chemostat setup is given in Krujatz et al. (2015). In this study, the PBR was operated at constant average surface illumination intensity (I_{ave}) of 2250 W m^{-2} (Figure 1b). The initial batch cultivation conditions were pH 7.3, 30 ± 0.5 °C, stirrer speed of 550 rpm and a bacterial load (dry weight) of 0.05 g L^{-1}. In case of chemostatic operation, the peristaltic pumps were switched on after 18 hours of batch operation to maintain a dilution rate of 0.1 h^{-1}. After reaching steady-state conditions in chemostatic operation (ca. 80 hours), the effects of varying the stirrer speed (at 150, 300, 600 and 750 rpm) on the bacterial dry weight (c_{dw}) and rate of hydrogen production (rH_2) was investigated. The c_{dw} and rH_2 analytics were performed as previously described (Krujatz et al., 2015).

Calculation of the stirrer Reynolds number

Assuming that the viscosity of the fluid is equal to that of water because of the low bacterial density in the medium, the stirrer Reynolds number (Re) was calculated according to Noorman (2006):

$$Re = \frac{\rho * N * D^2}{\eta} \quad (1)$$

Here, ρ and η are the density (995.65 Kg m^{-3}) and dynamic viscosity (0.0008 Kg m^{-1} s^{-1}) of water at 30 °C, respectively. N represents the stirrer speeds according to table 1 (e.g. 2.5 s^{-1}) and D the stirrer diameter (0.045 m).

ZEMAX - optical ray tracing simulation

ZEMAX is an optical design program that allows the optimization of illumination systems by ray tracing through optical elements such as lenses, mirrors or diffractive optical elements. It was used here to simulate the distribution of the intensity of illumination by the light sources (12 halogen spotlights), and to quantify the average illumination intensity (I_{ave}) available for the cells, inside the PBR. The spotlights were modelled using the ZEMAX knowledge base model (http://kb-en.radiantZemax.com). The electrical power consumption of each spotlight was 50 W, yielding an optical power output of 7.1 W ± 0.1 W (according to measurements with a Maestro single channel energy meter, supplied by Gentec). In the simulation tool, this optical power was equally distributed to two filaments, analogous to the used light sources. The reactor geometry was defined as a glass tube. A hexacontatetragonal virtual detector was placed at the inner reactor wall to represent the bacterial suspension (Figure 2). A million rays per filament were virtually emitted towards the transparent PBR vessel. By recording the amount and location of rays passing through the PBR wall and hitting the detector, the simulated I_{ave} and local illumination intensity distribution could be defined. The simulated illumination intensity distribution at the inner surface of the transparent PBR vessel was experimentally validated by measuring the intensity via an encapsulated pyranometer (Deka Sensor und Technologie, Teltow, Germany) which is sensitive to wave lengths between 350 nm and 1100 nm matching the spectral emission range of the halogen light sources.

Empirical light modelling approach for a cylindrical vessel with a bacterial suspension

The incident illumination intensity entering the transparent PBR vessel towards the bacterial suspension was attenuated by absorption and scattering at the cells. Each biological system has unique attenuation properties based on pigment composition and morphological cell properties (Murphy and Berberoglu, 2011). Approaches to analyze the influence of these individual properties can be divided into two major groups: deterministic and empirical. Since deterministic models entail high computational costs (Pottier et al., 2005), empirical methods are more suitable for the development of simple simulation tools.

The Beer-Lambert absorbance law utilizes one empirical parameter, as it solely describes attenuation by absorption. Thus, it is not applicable for modelling light profiles at high cell densities because the influence of scattering effects is neglected. To improve the empirical characterization, several approaches using two or three empirical parameters have been developed (Cornet et al. 1992; Cornet et al., 1995). In this study, a three-parametric hyperbolic model, introduced by Suh and Lee (2003), considering the attenuation of illumination intensity as a function of the length of ray trajectories and cell-specific light attenuation, was used to simulate illumination profiles within the bacterial suspension:

$$\frac{\mathrm{I}}{\mathrm{I_0}}(c_{dw}, \mathrm{l}) = \frac{R_0}{R} \exp\left[\frac{\varepsilon_\mathrm{m} * \mathrm{l} * \mathrm{c_{dw}}}{(\mathrm{K_c} + \mathrm{c_{dw}})(\mathrm{K_l} + \mathrm{l})}\right] \quad (2)$$

The hyperbolic equation includes the biomass dry weight concentration $\mathrm{c_{dw}}$ and the three empirical parameters ε_m, K_c, K_l, which have to be determined empirically at different ray trajectory lengths. The transparent PBR vessel is described by cylindrical coordinates r, φ, z. The light source coordinates Φ, Θ, R were translated to general coordinates assuming the position of the light source L to be r_L, φ_L, z_L:

$$\mathrm{r} * \cos\varphi * \sin\theta = \mathrm{r_L} * \cos\varphi_\mathrm{L} * \sin\theta_\mathrm{L} + \mathrm{R_L} * \sin\Phi * \cos\Theta \quad (3)$$

$$\mathrm{r} * \sin\varphi * \sin\theta = \mathrm{r_L} * \sin\varphi_\mathrm{L} * \sin\theta_\mathrm{L} + \mathrm{R_L} * \sin\Phi * \sin\Theta \quad (4)$$

$$r * \cos\theta = r_L * \cos\theta + R_L * \cos\Theta \quad (5)$$

By solving equations (3), (4) and (5) the light path length between a point r, φ, z and the light source r_L, φ_L, z_L, can be calculated. Indeed, a local intensity distribution also appears along z-axis. This point was simplified here by considering a distinct cross section of the reactor at the position of the installed stirrers to get a characteristic profile of illumination intensity at this position. To get a fully three-dimensional profile the simulation has to be performed either in a z-stack or the local intensity distribution along z-axis has to be described by an appropriate function I(z). In the assumed case, R_L (the sum of R_{in} and R_{out} in Figure 3) becomes a function of r and φ.

$$R_L(r, \varphi) = \sqrt{r^2 + r_L^2 - 2r * r_L \cos(\varphi - \varphi_L)} \quad (6)$$

Because the illumination intensity I_0 is a function of φ_R it has to be determined for every angle φ_R at the arched PBR surface (Figure 3). The intersection coordinates were calculated by geometrical considerations for every light source L and every position r, φ yielding a function $f_L(r, \varphi)$, which describes the fraction of $R_L(r, \varphi)$ inside and outside the PBR:

$$f_L(r, \varphi) = \frac{R_{L,in}(r, \varphi)}{R_{L,out}(r, \varphi)} \quad (7)$$

By introducing $f_L(r, \varphi)$ into equation 2, the path length of rays inside the bacterial suspension and the intersection with the PBR surface are defined:

$$I(c_{dw}, r, \varphi) = \frac{I_0(\varphi_R)}{f_L(r, \varphi) + 1} * \exp\left[\frac{\varepsilon_m * c_{dw} * R(r, \varphi) * (f_L(r, \varphi)^{-1} + 1)^{-1}}{(K_c + c_{dw})(K_l + [R(r, \varphi) * (f_L(r, \varphi)^{-1} + 1)^{-1}]}\right] \quad (8)$$

The total illumination intensity at a randomly chosen location r, φ is described as the sum of the local illumination intensities of every light source L:

$$I\,(c_{dw}, r, \varphi) = \sum^{L} \frac{I_0(\varphi_R)}{f_L(r,\varphi)+1} * \exp\left[\frac{\varepsilon_m * c_{dw} * R_L(r,\varphi) * (f_L(r,\varphi)^{-1}+1)^{-1}}{(K_c+c_{dw})(K_l+[R_L(r,\varphi) * (f_L(r,\varphi)^{-1}+1)^{-1}]}\right] \quad (9)$$

All visualizations of illumination intensity profiles within the bacterial suspension were performed using Mathcad 15.

Determination of empirical attenuation parameters ε_m, K_c, K_l

By using a converging lens to determine the cell-specific attenuation parameters of *R. sphaeroides* DSM 158 in a measurement chamber developed in-house (Figure 4a and 4b), the model simplified:

$$\frac{I}{I_0}(c, l) = exp\left[\frac{\varepsilon_m * l * c_{dw}}{(K_c + c_{dw})(K_l + l)}\right] \quad (10)$$

130 mL of bacterial suspension with dry weights ranging from 0.05 g L^{-1} to 3.0 g L^{-1} were placed in the chamber. An adjustable slide (manufactured in-house), carrying the above described encapsulated pyranometer was moved along the light path while monitoring the illumination intensity, thereby acquiring high-resolution data. A blank measurement was acquired to quantify the scattering caused by the side walls. The obtained data were used to calculate the attenuation parameters using multiple, non-linear regression in MATLAB 2012.

Computational Fluid Dynamics (CFD)

The fluid flow profiles and velocities induced by the three six-bladed Rushton stirrers were simulated using the commercial software COMSOL Multiphysics.

The rotating machinery interface of COMSOL Multiphysics allows the user to formulate the Navier-Stokes equations in a rotating coordinate system. A fluid flow simulation was performed using a mesh technique where the flow domain is divided into an inner rotating area and an outer stationary area coupled by a continuity condition according to Zhang (2013). To

reduce computing time, the reactor model was simplified by designing a flat bottom and neglecting small reactor components like the aeration pipe, sampling pipe and PT100 pipe. Within the rotating area and at the installed baffles the grid size was set at 0.0015 m. In parts of the reactor where small changes of fluid properties were expected a grid size of 0.00388 m was selected, resulting in 298,914 volume elements to solve. Given the anaerobic cultivation conditions (without exposure to gas), a single-phased system consisting of the fluid phase (water) was assumed. The fluid properties were defined as non-compressible, ρ=1000 kg m^{-3} and ν=0,001 Pas. The standard k-ε turbulence model was used to describe the fluid behavior. The two introduced parameters are k, the turbulent kinetic energy and ε, the turbulent dissipation rate. The transport equations for a standard k-ε model are given by:

$$\frac{\partial(\rho k)}{\partial t}+\frac{\partial}{\partial x_j}\left(\rho U_j k\right)=\frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_k}\right)\frac{\partial k}{\partial x_j}\right]+P_k-\rho\varepsilon \quad (11)$$

$$\frac{\partial(\rho \varepsilon)}{\partial t}+\frac{\partial}{\partial x_j}\left(\rho U_j \varepsilon\right)=\frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_k}\right)\frac{\partial \varepsilon}{\partial x_j}\right]+\frac{\varepsilon}{k}\left(C_{\varepsilon 1}P_k-C_{\varepsilon 2}\rho\varepsilon\right) \quad (12)$$

The turbulence viscosity μ_t is modeled as:

$$\mu_t=C_\mu\rho\frac{k^2}{\varepsilon} \quad (13)$$

The values for C_μ, σ_k, σ_k, $C_{\varepsilon 1}$ and $C_{\varepsilon 2}$ were (COMSOL standard settings):

$$C\mu=0.09 \qquad \sigma k=1.00 \qquad \sigma\varepsilon=1.30 \qquad C\varepsilon 1=1.44 \qquad C\varepsilon 2=1.92$$

Particle tracing

The "Particle Tracing Module" of COMSOL Multiphysics allows the computation of particles' trajectories in fluids and electromagnetic fields, including particle-particle, particle-field, and fluid-particle interactions. For fluid flow applications different forces are allowed to act on the particles: drag, gravity, dielectrophoretic and/or thermophoretic. The cells move under the influence of the fluid velocity field computed above through well-illuminated parts near

the reactor wall and slightly-illuminated zones in the center of the PBR, so each individual cell has a characteristic light/dark history. A time-dependent simulation of five seconds was run to analyze particle tracks within the fluid using different stirrer speeds.

The particle tracing equation in COMSOL is given as:

$$\frac{d}{dt}(m_p v_i) = m_p F_d (u_i - v_i) \quad (14)$$

where v_i describes the particle velocity and F_d the drag force. To calculate a spherical-equivalent d_p (≈1.02 µm) microscopic bright field measurements (WF Olympus, Tokyo, Japan) were performed to determine the length (1.1 ± 0.05 µm) and radius (0.4 ± 0.05 µm) of 20 cells. The cellular density was assumed to 1.0 g cm^{-3} which is in accordance to measurements of Sharma et al. (1993). The trajectories of 1000 particles induced by mixing were simulated assuming stirrer speeds of 300 and 750 rpm. The particle positions were converted to a radius-time-pattern and finally analyzed by Fast Fourier Transformation to obtain a typical illumination frequency at the prevailing bacterial dry weight within the PBR. The transformations were carried out using MATLAB 2012 according to Perner-Nochta and Posten (2007).

Results and Discussion

Effects of varying the stirrer Reynolds number on the rate of continuously produced hydrogen (rH_2) and dry weight (c_{dw}) in a chemostatic PBR

Since the steady-state conditions in a chemostat ensure that the biomass concentration and rate of product formation remain constant, the influence of various process parameters on productivity can be readily investigated. Here, steady-state conditions were maintained with a dilution rate of 0.1 h^{-1} and the stirrer Reynolds number (Re) was varied, by changing the speed of the installed Rushton impellers, resulting in flows ranging from laminar to highly turbulent, at a constant I_{ave} of 2250 W m^{-2}.

Figure 5 illustrates and Table 1 summarizes the results of varying the impeller speed on the attained c_{dw}, rH_2 and specific rH_2. The highest c_{dw} (2.26 ± 0.1 g L^{-1}) was obtained at 150 rpm, corresponding to a laminar fluid flow at Re = 6,300 (assuming a critical Re of 10,000). By increasing Re up to 31,600, which is associated with the transition towards turbulent flow conditions, the bacterial dry weight first slightly decreased but remained constant between 1.84 ± 0.02 g L^{-1} and 1.96 ± 0.02 g L^{-1} at increasing degrees of turbulence. The rate of product formation, rH_2, showed significant dependence on the prevailing Re. Doubling Re from 6,300 to 12,600 increased rH_2 by 17 % to 170.5 ± 2.2 mL L^{-1} h^{-1}. The further increase towards higher turbulence caused a reduction of rH_2 to 69 % of its maximum at a Re of 31,600. As Re was varied, by modulating stirrer speed, at a steady chemostatic state, changes in the concentrations of organic substrates could be excluded as reasons for the changes in rH_2. The bacterial dry weight changed only slightly with increasing turbulence, whereby it can be conclude that these results were direct consequences of changes in cell-specific illumination patterns associated with the changes in fluid flow.

In general, bacteria are not susceptible to shear stress because there dimensions are much less than the microscale of turbulence, especially in stirred tank reactors (Chisti 2009). To exclude that shear effects were responsible for the decrease of rH_2 at increasing turbulence a number of batch runs was performed at varying fluid flow conditions to investigate the influence on the maximum specific growth rate μ_{max} [h^{-1}]. The data provided in the supplementary files indicated no negative effect of stirrer speeds up to 750 rpm (Re = 31,600) on μ_{max} [h^{-1}]. In order to describe and quantify the changes in illumination conditions and performance of the externally illuminated chemostat PBR, a toolbox of simulations was developed.

Optical ray tracing simulation

The illumination intensity distribution and its average value (I_{ave}) throughout the surface of the PBR were analyzed by ZEMAX ray tracing simulation (Figure 2). To our knowledge, this

is the first application of ray tracing simulation for the optical characterization of an externally illuminated PBR. By simulating the ray trajectories of the illumination device, it was found that 75.8 % of the rays emitted by the lamps passed through the transparent PBR wall towards the bacterial suspension. Despite the small distance of only 5 cm between the light sources and PBR surface, 24.2 % of the emitted rays either missed the PBR vessel or were reflected at the glass surface because their angle of incidence exceeded the critical angle. Assuming that the 12 spotlights provided a total optical power of 85.2 W, a simulated I_{ave} of 1339 W m^{-2} for the inner surface of the cylindrical PBR was calculated by the simulation tool. The simulated intensity values were validated by measuring the local illumination intensity inside of the transparent PBR vessel at a reactor height of 80 mm (Figure 6). Due to the size of the used pyranometer experimental data showed a significant lower resolution compared to ray tracing simulation. However, there is an identical trend of simulated and experimental values whereas intermediate sections have been highly underestimated by the simulation tool. Consequently, the simulated I_{ave} for the whole surface of the PBR (1339 W m^{-2}) and experimental determined I_{ave} (2250 W m^{-2}) differed significantly. The deviation originates from difficulties in determining the geometry of the commercially available lamps, particularly the position of the tungsten filament in the bulb, and manufacturing variations among the individual lamps that cannot be considered in the simulation. In addition to assessing I_{ave}, this simulation tool was used to visualize the illumination intensity distribution at the virtual detector representing the bacterial suspension. As shown in Figure 6, there were distinct peak intensities at the positions of the 12 light sources, in accordance with expectations due to the parabolic reflectors of the halogen spotlights. The lowest illumination intensity was just 10 % of the peak intensity. This illustrates the importance of recognizing the intensity distribution, as cells are exposed to widely varying illumination when moving both from self-shaded dark regions in the center of the PBR to the well-illuminated PBR periphery, and even between well-lit and relatively poorly lit positions close to the PBR wall.

In the only previous published study in which optical ray tracing simulation of a PBR system was used, Zijffers et al. (2008) quantified the amount of captured sunlight in an outdoor optical fiber-equipped algal reactor. A key advantage is that the distribution of illumination intensities can be visualized, thereby improving understanding of the process conditions to which the microorganisms are exposed. The information about the "illumination yield", i.e. how much of emitted energy has reached the photosynthetic cells, can be further used for energy balances. Concluding, though the ray tracing simulation cannot describe the optical setup highly accurately, quantitative predictions of illumination intensities, and especially their distribution, can be estimated and visualized by optical ray tracing.

Empirical simulation of illumination intensity attenuation within bacterial suspensions

The empirical attenuation parameters ε_m, K_c and K_l were determined by fitting equation 10 to experimental data (Figure 4c), using MATLAB 2012, with a R^2 of 99.54. The obtained parameters (0.05559, 1.21 g L^{-1} and -51.51 m, respectively) were used to simulate the attenuation of illumination intensity within the cylindrical PBR. Figure 7 illustrates the prevailing illumination conditions at the beginning of the batch phase (c_{dw} = 0.05 g L^{-1}, Figure 7a). To simulate the illumination distribution at steady-state a c_{dw} of 2.0 g L^{-1} was assumed (Figure 7b). At a low cell density, the intensity of the light sources cumulates in the center of the reactor, causing a local illumination intensity maximum. Due to the low self-shading, the cells are provided with high illumination intensity across the entire cross-sectional area of the PBR. At steady-state bacterial cell dry weight (Figure 7b), the illumination intensity becomes strongly attenuated due to absorption, self-shadowing and scattering. The bacterial cells are only provided with sufficient illumination intensity within the first centimeter of the PBR periphery. Consequently, to maintain the productivity of the process, bacterial cells have to be actively transported to the reactor surface to absorb photosynthetic active radiation (PAR). The PAR of PNS bacteria is composed of radiation between 400 nm – 500 nm (carotenoids) and 750

nm – 900 nm (bacteriochlorophyll a). 21.8 % of the total halogen emission spectrum is PAR. Because no filter systems were used to reduce the illumination intensity to PAR the measured whole spectrum data were used for the light profile simulations.

Since both light scattering and cell-specific attenuation of illumination intensity are accounted for by the three-parametric hyperbolic equation, the distribution of illumination intensity within the cylindrical PBR should have been reasonably realistically simulated. More complex mathematical formulae can be used to improve the description of light profiles in PBRs. For instance, Csögör et al. (2001) used a statistical-numerical (Monte-Carlo) simulation approach to calculate trajectories of photons through bacterial dispersions. However, a large number of parameters have to be considered and experimentally validated for such numerical approaches, e.g. absorption, refraction and scattering of photons at various surfaces, air bubbles and cells. Considering the complexity of the models and difficulty of acquiring sufficiently high-resolution data for such approaches, the use of empirically-based models is recommended.

Characterization of fluid flow patterns by Computational Fluid Dynamics

Common PBR setups that use pneumatic power inputs for mixing (e.g. flat panel, bubble column and airlift reactors) would be inconvenient for cultivating facultative or obligate anaerobic phototrophic microorganisms since sparging of an inert gas would be required. Hence, anaerobic phototrophs are normally cultivated in externally illuminated laboratory bottles or test tubes sealed with gaskets or a rubber-stopper (Wang and Wan, 2009), which prevents (or at least severely hinders) monitoring and control of the process. Despite having a lower surface to volume ratio than flat panel or tubular reactors, stirrer-mixed PBRs can provide reasonable alternatives for preventing sedimentation of cells under anaerobic conditions. In addition, the fluidal flow pattern can be directly influenced by the type of stirrer, and both the trajectories

of cells and their residence times in well-illuminated parts of the PBR can be actively influenced. Thorough consideration of fluid dynamics and particle-fluid-interactions is essential for understanding and optimizing the performance of a PBR.

Flow structures and fluid velocities

Due to the symmetry of cylindrical vessels the geometry of the STR was reduced to a two-dimensional simulation area, a method which is a common approach to reduce computing time (Zhang 2013; Jenne and Reuss 1999). Lestinsky et al. (2012) found a good correlation between simulated and experimental fluid velocity data using a COMSOL Multiphysics generated mesh with grid sizes between 0.001 m and 0.007 m depending on the expected fluid velocity gradients in an airlift reactor. In this study, we iteratively adapted the grid sizes for the different parts of the STR until a sufficient convergence and computing time could be realized. The final grid structure (grid size 0.0015 m – 0.0038 m) is available in the supplementary files.

The simulated flow patterns for the PBR setup used in this study are illustrated in Figure 8. Three six-bladed Rushton stirrers provided a radial flow of the fluid towards the well-illuminated PBR periphery. As might be expected, the fluid velocity was found to be maximal at the stirrer blades. A stirrer speed of 150 rpm generated a maximum radial fluid velocity (v_{maxr}) of 0.3 m s^{-1}. This result for the fluid flow velocity is in accordance with simulated and experimental validated data of Costes and Couderc (1988) who measured a v_{maxr} of ca. 0.3 m s^{-1} at 165 rpm in a similar STR by laser doppler anemometry. At 150 rpm there were also poorly-mixed zones ($v \leq 0.1$ m s^{-1}) in upper and lower parts of the cylindrical vessel, as well as intermediate zones between the stirrers. Increasing the stirrer speed to 300, 600 and 750 rpm increased v_{maxr} to 0.6 m s^{-1}, 1.3 m s^{-1} and 1.6 m s^{-1}, respectively. The fluid patterns simulated at increasing stirrer speeds indicate a tangential movement to the top and bottom of the cylindrical vessel, enhancing the degree of mixing. This also increased the fluid velocity in poorly-

mixed zones to $v \geq 0.4$ m s^{-1}. Similar fluid velocities have been reported; 0.477 m s^{-1} in a stirrer-mixed torus-shaped PBR (Pruvost et al., 2006; Pruvost et al., 2008) and 0.5 m s^{-1} in a bubble column used for cultivating algae (Nauha and Alopaeus, 2013).

Due to the good agreement of simulated data in this work with experimental and simulated data of the above mentioned studies we assess the COMSOL generated mesh with grid sizes between 0.0015 m to 0.0038 m sufficient to get a reliable fluid velocity simulation within acceptable length of computing time (≈ 5 days). The standard k-ε turbulence model has been successfully applied in a large number of studies, whereby also limitations in accuracy, especially for the description of recirculation loops and in comparison to experimental data have been reported (Zhang 2013). Therefore, the k-ε model was extended or modified to improve the accuracy of fluid flow simulations, e.g. Chen-Kim k-ε model or RNG k-ε model. However, the standard k-ε model is computational stable, can be applied for a wide variety of turbulent flows and is implemented in the commonly used CFD software tools.

Data processing and particle tracing

Particle tracing was described by Ali et al. (2015) as a novel technique to estimate the behavior of cells within a PBR by numerical computation. The rate of hydrogen production, rH_2, was directly influenced by the prevailing fluid flow pattern. The trajectories of 1000 particles (bacterial cells) and typical illumination frequencies were analyzed under the conditions providing the highest (300 rpm) and lowest (750 rpm) productivity using the COMSOL "Particle tracing" module. Figure 9a and 9b illustrate the cyclic trajectories of the bacterial cells, between the center and PBR periphery, induced by the rotating stirrers. The typical illumination frequency was determined, by Fast Fourier Transformation following Perner-Nochta and Posten (2007), to 1.5 Hz and 5.5 Hz at 300 rpm and 750 rpm, respectively. ~~Contrary to expectations,~~ the productivity decreased at the higher average illumination frequency of 5.5 Hz (750 rpm). This result could be caused by two effects: First, at increasing turbulence the fluid flow

in the whole PBR volume became more undirected, i.e. the cells were less forced in distinct trajectories across the PBR radius compared to a lower stirrer speed and Reynolds number which could negatively affect the light supply of cells. Second, the high frequency of high intensity illumination at 750 rpm probably caused photoinhibitory effects. This hypothesis is substantiated by the slightly decrease of bacterial dry weight concentration at increasing turbulence. A shear stress effect was excluded as indicated in the supplementary material. To conclude, at high external illumination intensity the productivity of continuous hydrogen-producing PNS bacteria was directly influenced by the fluid flow and subsequent frequency of illumination. In this study, an average illumination frequency of 1.5 Hz at low turbulence (Re = 12,600) induced by a radial fluid flow in a stirred PBR was identified as optimum conditions for *R. sphaeroides* DSM 158 for this PBR setup.

Conclusions

An often neglected but permanently present challenge in PBR characterization are the highly heterogeneous illumination conditions of photosynthetic cells during their trajectories across a PBR due to the high mutual self-shading and complex optical effects. However, if the fluid dynamics are addressed this phenomenon can be exploited to actively regulate the cell-specific illumination conditions. It is also important to obtain a thorough understanding of the prevailing illumination conditions to accurately assess photobiotechnological processes. We have demonstrated that considering all parameters (paths, distributions and cellular trajectories) influencing the "abiotic factor" light is valuable for optimizing continuous photoheterotrophic hydrogen production in a stirred PBR, and by doing so we have obtained high rates of hydrogen production using *R. sphaeroides* DSM 158.

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Competing interests

The authors declare that there are no competing interests.

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Figure legends

Figure 1: Laboratory photobioreactor setup. (A) The Applikon 1 L stirred tank reactor equipped with three six-bladed Rushton impellers, (B) Laboratory setup: STR reactor with illumination device containing 12 halogen spotlights.

Figure 2: ZEMAX optical ray tracing simulation. (A) Photobioreactor setup with cylindrical transparent vessel and the hexacontatetragonal virtual detector (striped cylinder) representing the bacterial suspension. (B) ZEMAX ray tracing simulation, showing eight rays per simulated filament of the halogen light sources.

Figure 3: Schematic diagram of a horizontal section of the cylindrical photobioreactor vessel. Light trajectory R from the external light source L to a random spot P within the reactor is the sum of the light paths inside the bacterial suspension R_{in} and outside R_{out}. Incident intensity I_0 is determined at the angle φ_R of the intersection between the bacterial suspension and light trajectory.

Figure 4: Determination of empirical cell-specific light attenuation parameters of *R. sphaeroides* DSM 158. (A) CAD-construction of the measurement chamber consisting of (1) halogen light source, (2) converging lens, (3) movable silicon photodiode, (4) measuring chamber containing bacterial suspension. (B) Experimental determination for different dry weight concentrations of *R. sphaeroides* DSM 158 and light path lengths. (C) Experimental results, black balls represent measurement points whereas the area plot is the result of multiple non-linear regression to determine the cell-specific attenuation parameters using MATLAB 2012.

Figure 5: Effects of varying the Reynolds number on the rate of hydrogen production and dry weight. *Rhodobacter sphaeroides* DSM 158 was cultivated in an externally illuminated chemostatic PBR at steady-state conditions. Striped-line indicates the transition from laminar to

turbulent fluid flow (assumed critical Reynolds number of 10,000). Error bars are the result of averaged values over 24 hours steady-state operation.

Figure 6: (A) Illumination intensity distribution at the virtual detector (representing the bacterial suspension) visualized by ZEMAX optical ray tracing simulation for the PBR setup. (B) comparison of simulated and experimental determined intensity data at a reactor height of 80 mm.

Figure 7: Simulated light profiles within the cylindrical PBR vessel at (A) 0.05 g L^{-1} biomass at initial batch conditions and (B) 2.0 g L^{-1} biomass at chemostatic steady-state conditions.

Figure 8: CFD-simulation of fluid flow pattern, fluid velocities and flow directions. The simulations were performed for varying stirrer speeds (150, 300, 600 and 750 rpm) of the three six-bladed Rushton impellers.

Figure 9: Particle tracing simulation. Typical particle trajectories at stirrer speeds of (A) 300 rpm and (B) 750 rpm. The simulation was performed by COMSOL particle tracing. The dotted line represents the boundary between well-illuminated (reactor periphery) and slightly-illuminated zones at a steady-state c_{dw} of 2.0 g L^{-1}.

Table 1: Overview on experimental results during chemostat cultivation. Summary of attained bacterial dry weights, hydrogen production rates and specific hydrogen production rates of *R. sphaeroides* DSM 158 at the applied stirrer speeds during steady-state conditions. Error bars are the result of averaged values over 24 hours steady-state operation.

Commented [JW1]: Standard deviations?

Stirrer speed [rpm]	Reynolds number [-]	Max. radial fluid velocity [m s^{-1}]	Bacterial dry weight [g L^{-1}]	Hydrogen production rate [mL L^{-1} h^{-1}]	Average specific hydrogen production rate [mL g^{-1} h^{-1}]	Average molar specific hydrogen production rate [mmol g^{-1} h^{-1}]*
150	6,300	0.3	2.26 ± 0.10	142.0 ± 4.2	62.8	2.57
300	12,600	0.6	1.96 ± 0.01	170.5 ± 2.2	87.0	3.56
600	25,300	1.3	1.94 ± 0.04	146.6 ± 2.4	75.6	3.09
750	31,600	1.6	1.84 ± 0.02	117.5 ± 2.3	63.9	2.61

* assuming standard ambient temperature and pressure conditions (25°C, 1013 hPa)