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Development of a Semi-Theoretical Light Radiation and Photosynthetic Growth Model for the Optimal Exploitation of Wastewaters by Microalgae

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In the last decade, interest toward the potential application of microalgae has grown considering their potential use in industrial sectors as human nutrition and health, animal feed and biopolymers. Their ability to use light or/and organic carbon as energy source, makes them able to grow in a wide range of conditions. Because of that, the possibility to use alternative nutrients and water sources for their cultivation has been investigated. The microalgal cultivation using wastewaters mixed with synthetic medium might be a good combination that could reduce costs of water, nutrients and wastewater treatment. Anyway, wastewaters are frequently dark colored and contain toxic compounds that could have a negative impact on microalgal light uptake and metabolism. In this study, an experimental-first principles hybrid method for the estimation of microalgal growth in non-transparent media was developed as a guide in the choice of the best formulation of wastewater-based culture media for microalgae. To carry out several experimental runs in parallel with different conditions (dilution of the wastewater, different light sources, etc.) a cylindrical bubble column PhotoBioReactor (PBR) was adopted. Its simple geometry allows the analysis of inside light fluxes. A non-metabolizable and non-toxic dye, in condition of purely light-radiative growth limitation, was added to the medium mimicking the reduced transparency of wastewaters. As final step to test the model, culture mediums with wastewater addiction were used for microalgal cultivation, showing their nutritive effects on growth.

1. Introduction

Microalgae are a source of high added value products with potential applications in the food, cosmetic and pharmaceutical industries (Sed et al., 2017). Anyway, microalgal cultivation in phototrophic ways presents still problems of high costs. The use of wastewaters as nutrients source for microalgal cultivation may reduce the culture management and water treatment costs but promotion and demotion effects complicate data analysis and exploitation (Gouveia et al., 2016). An hybrid approach to data analysis, coming out from the post-treatment of experimental data with a semi-theoretical light-centered microalgal growth model, enabled the split of metabolic effects due to dissolved substances (substrates, nutrients, toxins) from that of light (considered as a substrate). Furthermore, an improvement to the previous work (Cicci et al. 2014b) for a better data interpretation has been added, considering light-scattering phenomena and introducing an absorption efficiency calculated in all wavelengths (λ) of visible spectrum. The model would allow the microalgal process developer to find the ideal composition of a wastewater-incorporating medium at the optimal scale for laboratory array testing. In the present work, some models of irradiance distribution (Kumar et al. 2013, Cornet et al. 1995) and phototrophic growth (Monod et al. 1949, Pruvost et al. 2002), with different level of accuracy and complexity, were considered. Combination models were benchmarked for their ability to describe the effect of light supply reduction on observed specific growth rate adopting the concept of semi-theoretical normalized growth rate. The best performing models came out from the combination of the mono-dimensional approach of the Radiative Transfer Equations described in the Two-Flux model with the growth model described by Pruvost et al. (2002). However, a satisfactory compliance with the obtained experimental

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results also required the analysis of the irradiance effects at a spectral level, i.e., taking into account the absorption efficiency of microalgae and the photosynthetic action at each wavelength in the (photosynthetically active) spectrum. The experimental growth rate obtained in light limitation condition inside the photobioreactor, using first a dye simulating non transparent water, and then wastewaters mixed with a synthetic medium (Olive Oil Mill WasteWater and digestate) were used to test and validate the developed model (Cicci et al. 2014a).

2. Materials and methods

2.1 Microalgal cultivation and dye addition.

Scenedesmus dimorphus (UTEX 1237) was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA. It was inoculated into a Bold 3N modified (3NB) culture medium; a liter of medium contains: 0.025 g NaCl, 0.75 g $NaNO_3$, 0.075 g $MgSO_4*7H_2O$, 0.075 g K_2HPO_4, 0.175 g KH_2PO_4 , 0.019 g $CaCl_2$ and 6 mL of PVI metal solution composed by: 0.7 g/L of $Na_2EDTA*2H_2O$, 0.097 g/L of $FeCl_3*6H_2O$, 0.041g/L of $MnCl_2*4H_2O$, 0.005 g/L of $ZnCl_2$, 0.002 g/L of $CoCl_2*6H_2O$, 0.04g/L of $Na_2MoO_4*2H_2O$. It was sterilized by autoclaving at 121 °C for 20 minutes. A black water-based color (Pigment PBk6, PBk7, vehicles Arabic Gum) was first diluted in distilled water and after sterilized; S. Dimorphus 1237 is unable to metabolize Arabic gum. The series of colored media was prepared by diluting the mother solution in synthetic medium to a final absorbance of: 0.1, 0.2, 0.4, 0.6, and 0.8 measured at 690 nm. The cultures were grown in a Polymethyl methacrylate (PMMA) cylindrical photobioreactors with a volume of 500 mL and a diameter of 6 cm, insufflated with filtered and pre-humidified air to prevent evaporation. Air flowrate was set at 5.4*10-3 vvm (130 NL/ h) to gain also a good mixing. These photobioreactors were kept in a climatic cabin where lighting was provided by eight cold white fluorescent lamps (400-700 nm, 865 K, 32 W, 80 µmol photons*$m^{-2}s^{-1}$) with a light/dark period of 16:8 hours. Temperature inside the cultures was maintained constant at 23 ± 2 °C. The wastewaters used in substitution of dyed medium were: a cattle digestate from a plant located in Cicerale (Southern of Italy); OOMWW (Olive Oil Mill WasteWater) from a olive oil plant located in Sabina area (Center of Italy). The digestate was sieved at a 710 µm aperture to get rid of suspended solids and micro-filtered in a polyamide column (type JX) (pore size 0.3 µm), then it was thermally sterilized (20 minutes at 120 °C) (Cicci et al. 2014a). The raw OMWW was sieved at a cut-size of 300 micron, then it was flocculated by acidification with nitric acid to pH values near 3.0, centrifuged and photo-catalyzed (Cicci et al. 2013).

2.2 Microalgal harvesting and growth monitoring

The cultures were carried on for a semi-continuous growth time of 17 days. At the end, the biomass was collected, centrifuged and washed twice with distilled water and dispatched for chemical assays. Afterwards the collected algae were transferred to a dehumidified flask and placed for one night to dry in an oven at a temperature of 110 ° C. The dried biomass was used to calculate the dry weight net of the tare. The relationship found between cell density C (g/l) and absorbance measured at the wavelength of 690 nm corresponds to the linear correlation:

$$C = 0.92 * A_{690} \qquad \text{with} \quad R^2 = 0.99 \tag{1}$$

The absorbance measures were collected with a spectrophotometer UV-1800PC (Shanghai Mapada Instruments Co., Ltd).

3. Light and growth modelling

3.1 Introduction to the general method of analysis

Before introducing models for growth and light distribution, the general method of data analysis already used in the work of Cicci et al.(2014b) is described. In order to separate the nutritional/toxic effects (e.g. wastewaters medium) from the energetic ones (e.g. illumination source) on microalgal growth, the irradiance inside the cylindrical photobioreactor was expressed as a function of the radial position I(r). Afterward, the instantaneous specific growth rate (μ_m) was calculated by integration of µ(r) in all volume of reactor, making it only dependent on the radial position after substitution of the irradiance function within it:

$$\mu_m = \int_0^R \mu(r) 2\pi r dr \tag{2}$$

The average specific growth rate (semi-theoretical) at a certain dilution ratio (D) is obtained by dividing the Eq (2) for the reactor's volume:

$$\bar{\mu} = \mu_{ST}(D) = \frac{\int_0^R \mu(r) 2\pi r dr}{\int_0^R 2\pi r dr} \tag{3}$$

This estimated growth rate it also normalized by dividing it for the estimated value of the growth rate at infinite dilution in the same medium without the dye ($\mu_{ST}(Ctr)$):

$$\mu_{STN} = \frac{\mu_{ST}(D)}{\mu_{ST}(Ctr)} \quad (4)$$

With the same procedure, the value of experimental growth at the average absorbance of the culture (medium + microalgae) was determined, assuming the cultural medium absorbance (A_{CM}) to be constant along the culture lifetime. The average absorbance, with these assumptions was calculated as:

$$\overline{A} = A_{CM} + \frac{A(t=0)+A(t)}{2} \quad (5)$$

Obtaining a normalized experimental growth rate:

$$\mu_{SPN} = \frac{\mu_{SP}(D)}{\mu_{SP}(Ctr)} \quad (6)$$

Finally, the relative growth rate (μ_R) was calculated, by dividing Eq. (6) by Eq. (4):

$$\mu_R = \frac{\mu_{SPN}}{\mu_{STN}} \quad (7)$$

Our aim was to demonstrate, with the help of a mathematical model, that in the normal experimental conditions with a dyed culture medium without surplus of nutrients ($\mu_{SPN}>\mu_{STN}$) or toxic elements ($\mu_{SPN}<\mu_{STN}$) in the medium, μ_R=1 will be obtained. This method provides the microalgal process prospector with a suitable data analysis tool to choose the "ideal" composition (Dilution Ratio) of a wastewater in microalgal cultivation with a limited set of small-scale experiments.

3.2 Growth model

The model used for representing the growth in function of irradiance is the 'Light-inhibition model' described by Pruvost (Pruvost et al. 2002):

$$\mu(I) = \frac{2\mu_s(1-\rho_c)(\rho-\rho_c)}{(1-\rho_c)^2+(\rho-\rho_c)^2} \quad (8)$$

With $\rho = \frac{I}{I_S}$, $\rho_C = \frac{I_C}{I_S}$, and μ_s that are in order: the normalized light intensity, the normalized light intensity compensation and the specific growth rate at saturation light (I_S); the values of which were assume to be: $I_C = 10\frac{\mu E}{m^2 s}$, $I_S = 150\frac{\mu E}{m^2 s}$ (Liu et al. 2013). The Pruvost model expresses the main aspects of the photosynthetic response of the microalgae to light, namely, the photoinhibition of the cells to high values of I, and the weight loss that occurs below a certain irradiance value, called compensation irradiance I_C.

3.3 Light distribution model

For light distribution inside the reactor two models were analyzed, both considering scattering phenomena inside the reactor. The first one is an empiric model obtained with the image analysis used as a tool in studying the light distribution, (Kumar et al. 2013). The irradiance function written in Eq(9) is actually a modified Lambert-Beer equation:

$$I(r,t) = \frac{R\cdot I_0}{R-r} e^{\frac{-\varepsilon\cdot C\cdot r}{(K_C+C)(K_R+r)}} \quad (9)$$

where $\varepsilon = 23.86$, $K_C = 4.03\ g/l$, and $K_R = 3.78\ cm$ are respectively: coefficients of maximal light absorption, light scattering by cells, and light scattering by light path-length taken from the quoted paper (Kumar et al. 2013); besides C is the cell density (g/l) inside the reactor. The second one is a more rigorous model, based on the solution of the Radiative Transfer Equations (RTEs), which can be derived by considering a small element of culture suspension and performing a radiation balance on this element, considering the incident, absorbed, transmitted, and scattered sources of radiation. The solution of RTEs in its tridimensional form is an integro-differential problem in a six-dimensional Euclidean space needing sophisticated numerical methods, so Cornet (Cornet et al. 1995) introduced a monodimensional approximation, called 'two flux approximation', splitting the field of radiation into two opposite and different fluxes I_r^+ and I_r^-. These considerations were applied to the cylindrical geometry used in the experiments and the flux balance obtained is schematized in Figure 1.

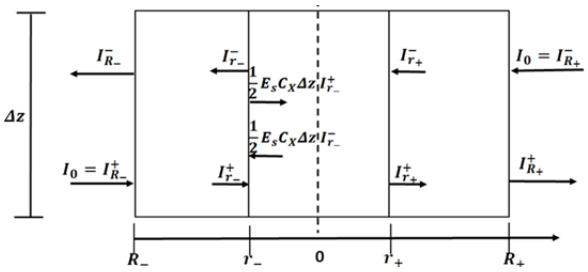

Figure 1: Flux balance schematization in cylindrical geometry applying the two flux approximation; $E_s$is the scattering parameter ($\frac{m^2}{kg}$) and I_0 is the incident irradiance ($\frac{W}{m^2}$).

In Figure 1 a back scattering coefficient $\mathrm{b} = \frac{1}{2}$ is considered because, after simulations at different b (data not shown), the Isotropic Scattering has been chosen as the best solution to represent the experimental light conditions; indicating that half of the irradiance follows the initial path and the other half is scattered in the opposite direction. The flux balance is summarized in Eq(10), where $\mathrm{C_X}, \mathrm{E_a}, \mathrm{E_s}$ are in order: the cell density inside the reactor ($\frac{\mathrm{kg}}{\mathrm{m^3}}$), the mean absorption and the scattering mass coefficients on the visible spectrum ($\frac{\mathrm{m^2}}{\mathrm{Kg}}$).

$$\begin{cases} \mathrm{I_r^+ 2\pi r\Delta z|_r - I_r^+ 2\pi r\Delta z|_{r+\Delta r} + \frac{1}{2}E_sC_XI_r^- 2\pi r\Delta r\Delta z|_r - \frac{1}{2}E_sC_XI_r^+ 2\pi r\Delta r\Delta z|_r = E_aC_XI_r^+ 2\pi r\Delta r\Delta z|_r} \\ \mathrm{I_r^- 2\pi r\Delta z|_r - I_r^- 2\pi r\Delta z|_{r+\Delta r} + \frac{1}{2}E_sC_XI_r^+ 2\pi r\Delta r\Delta z|_r - \frac{1}{2}E_sC_XI_r^- 2\pi r\Delta r\Delta z|_r = E_aC_XI_r^- 2\pi r\Delta r\Delta z|_r} \end{cases} \quad (10)$$

The Eq (10) after some simplifications gave the system that was actually implemented and solved in MATLAB (Eq(11)) using the function `bvp5c`, that is a finite difference code that implements the four-stage Lobatto III[a] formula to solve 'Boundary Value Problems'.

$$\begin{cases} \frac{\mathrm{dI_r^+}}{\mathrm{dr}} = -\frac{1}{\mathrm{r}}\mathrm{I_r^+} - \mathrm{E_aC_XI_r^+} + \frac{1}{2}\mathrm{E_sC_X}\,(\mathrm{I_r^-} - \mathrm{I_r^+}) \\ \frac{\mathrm{dI_r^-}}{\mathrm{dr}} = -\frac{1}{\mathrm{r}}\mathrm{I_r^-} + \mathrm{E_aC_XI_r^-} + \frac{1}{2}\mathrm{E_sC_X}(\mathrm{I_r^-} - \mathrm{I_r^+}) \end{cases} \quad (11)$$

With the relevant Boundary Conditions:

$$\begin{cases} \mathrm{r = R_-}\ \mathrm{I_r^+ = I_0} \\ \mathrm{r = R_+}\ \mathrm{I_r^- = I_0} \end{cases} \quad (12)$$

3.4 Absorption Efficiency introduction

For a better representation of the mechanisms that occurs in the phototrophic microorganisms, the considerations done by Senger (1970) were studied, and two new parameters were introduced: the Quantum Yield Spectrum (Φ=moles of O_2 produced/moles of absorbed photons), and the Action Spectrum (Ψ=moles of O_2 produced/moles of incident photons). With these preconditions a new parameter named 'Absorption Efficiency' was introduced (Eq (13)),

$$\mathrm{E} = \frac{\Psi}{\Phi} = \frac{\text{moles of absorbed photons} * \text{moles of } \mathrm{O_2} \text{ produced}}{\text{moles of incident photons} * \text{moles of } \mathrm{O_2} \text{ produced}} = \frac{\text{moles of absorbed photons}}{\text{moles of incident photons}} \quad (13)$$

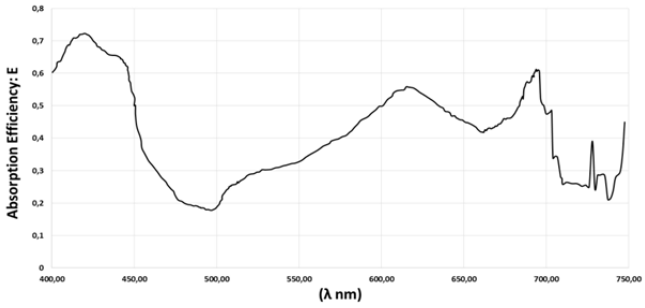

Figure 2: Absorption Efficiency calculated in all wavelengths (λ) of visible spectrum for the microalga Scenedesmus obliquus.

In addition to that, the Absorption Efficiency was calculated in all wavelengths of visible spectrum by data obtained from Senger's work (Senger 1970) (Figure 2), adding its effect to the absorbance of microalgae during the growth without the value of the dye, because this parameter affects only the algae themselves. At last, the value of the dye's absorbance was added to the value of absorbance modified with the Absorption Efficiency, considering also the coloring effects on microalgal growth.

4. Results

4.1 Application to artificial colored medium

Taking the above considerations into account, the results achieved by applying the previously described models to the growth data obtained with a balanced medium conditioned by a non-toxic and non-metabolized dye were plotted (Figure 3). It can be seen that the Cornet light model, associated with the Pruvost growth model predicts the experimental growth rate significantly better than the Modified Lambert-Beer model, although at the price of a higher complexity. Therefore, the Cornet and Pruvost models will be considered as the reference models to use for the future application of the model in predictive mode.

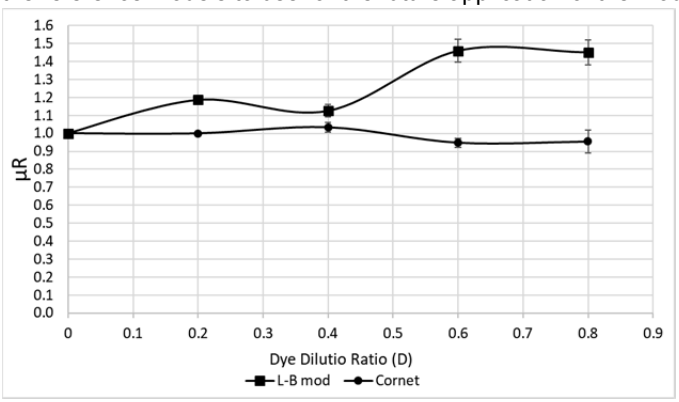

Figure 3: Relative specific growth rate μ_R in function of dye dilution ratio for both light distribution models: Two flux model(Cornet et al. 1995) and Modified Lambert Beer (Kumar et al. 2013). Both light models were coupled with the same growth model described by Pruvost (Pruvost et al. 2002).

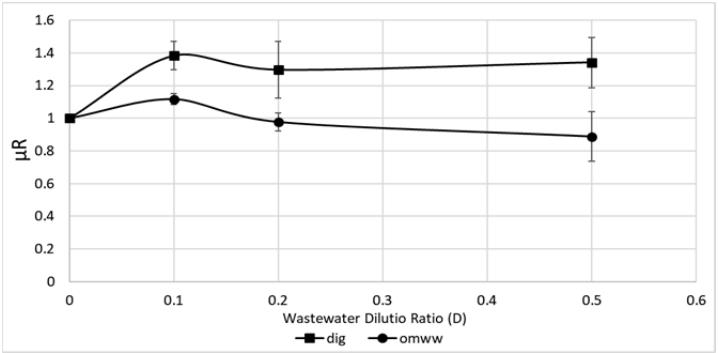

Figure 4: Relative specific growth rate μ_R in function of wastewater dilution ratio for both wastewaters: digestate(dig) and olive oil mill wastewater(oomww).

4.2 Application to wastewaters

As further implementations of this work, two types of wastewaters in substitution of the inert dye were used, showing their positive ($\mu_R > 1$) or negative ($\mu_R < 1$) effects on growth. The aim of this part of the work was to find the ideal composition (or dilution) of a wastewater-incorporating medium, coincident to the highest value of μ_R, using it as a base for a future tests without any further experimental analysis. The results are shown in Figure 4 both for the digestate and for OOMWW, and the positive effect of compounds (nitrogen and phosphorous) contributed by the wastewaters ($\mu_R > 1$ at all dilutions) can be seen particularly in the case of the digestate (Cicci et al., 2014a); for OOMWW the positive effects are less stronger revealing also a value less than one. It should be noted that this conclusion cannot be drawn upon the raw measured specific growth

rates only, which exhibit a continuous decrease of specific growth rate when the wastewater volume fraction in the formulated media is increased.

5. Conclusions

This work showed a method of analysis of the phototrophic microalgal growth inside a non-transparent media using a mathematical model that could be easily implemented and reused by any final users. This model incorporates all the principal phenomena that occur in a phototrophic cultivation, but also considers physical light phenomena like scattering that has been shown being indispensable for a proper representation of the problem. The results showed above confirmed the hypothesis made for a synthetic medium with an inert dye ($\mu_R \cong 1$), giving also positive response for using wastewaters in addition to growth medium. However the method needs to be studied more accurately with further specific tests in a wider range of dilution ratio, analyzing also the substitution, as diluent, of the synthetic media with tap water, reducing costs further.
In that case growth of the photobacteria would rely only on light and nutriments present in wastewater.
All of these considerations were done because wastewaters have many advantages in terms of diminishing treatment costs and raw material costs, but could also have to face with disadvantages like the presence of toxic elements for growth, and the model could help to preliminarily investigate that without too many experiments. To improve μ_{SP} and biomass yield, a new type reactor with an optimized geometry that diminish the microalgal thickness exposed to light could be designed (Moroni et al. 2014). This exposure has to be controlled, avoiding the photo-inhibition and fully exploiting the “metabolically compliant” medium formulated with the aid of the tool presented in this study.

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