Quantifying the potential of microalgae to remove nutrients from wastewater

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Abstract: The main resources limiting microalgae growth are typically phosphorus, nitrogen, and light. Based on the theory of the light limited chemostat, the variable cell quota approach, and photoacclimation models, we build a mathematical model for describing microalgae growth under limitation by these resources. The model is calibrated with a data set from the literature. Then, by numerical simulations, we find that under constant operation of the culture and constant environmental conditions (illumination, temperature, pH, etc.), solutions of the model approach towards either a positive or an extinction steady state. Based on the positive steady state, and in the context of wastewater treatment, we evaluate the capacity of microalgae to remove contaminants. We showed that the impacts of depth, incident light intensity, and dilution rate (or hydraulic retention time) have a crucial role on the optimization of the nutrient removal efficiency.

Keywords: Control; Biotechnology; Wastewater treatment; Microalgae; Cell quota model

1. INTRODUCTION

A major requirement in wastewater treatment is the removal of nutrients to acceptable limits prior to discharge and reuse. In this context, microalgae have shown a great potential for nitrogen and phosphorous removal in wastewater treatment (Abdel-Raouf et al. (2012); Sturm and Lamer (2011)). For this purpose, microalgae can be cultivated in high rate algal ponds (HRAPs). However, technical and economical constraints still exist for the implementation of full scale HRAPs for wastewater treatment. Among the challenges to be solved, maintaining an optimal working despite fluctuations in light, temperature, and influent nutrients is a key issue. For example, one problem with these systems is that light, one of the main factors affecting microalgae growth rapidly becomes limiting after its decrease due to absorption and scattering by algal cells (Morel and Bricaud (1981)) together with particulate matter and colored substances (Borowitzka (1998)). Mathematical models are of great help to better understand these nonlinear, dynamical and complex systems and finally to set-up control strategies.

Here, we propose a numerical study to determine the capacity of a HRAP to remove nitrogen and phosphorus from raw wastewater. In our approach, we build a mathematical model for describing microalgae growth under colimitation by light, nitrogen, and phosphorus, including photoacclimation dynamics. Nutrient limitation is described with the variable cell quota model proposed by Droop (1968). Thus, microalgae growth is related to intracellular pools of nitrogen and phosphorus known as cell quotas of nitrogen and phosphorus respectively. These cell quotas increase through absorption of nutrients from the growth medium. Light limitation is described using the theory of the light limited chemostat developed by Huisman et al. (2002). Thus, we consider the light gradient in the culture and the response of microalgae to the different light intensities in the growth medium. We also assume that microalgae may suffer from photoinhibition i.e. a decrease in the growth rate due to high light intensities. Photoacclimation dynamics is described with the model proposed by Bernard (2011). Thus, the response of microalgae to light depends

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on the chlorophyll content which varies with the light availability in the medium. The resulting model fits experimental data obtained by Wagner et al. (2018) for a batch culture with a microalgal consortium consisting mainly of Chlorella sorokiniana and Scenedesmus sp. Through numerical simulations, we study the long-term behavior of the trajectories of the model. Finally, we compute the removal capacities of the system for different hydraulic retention times and depths at steady state operation.

This article is organized as follows. In section 2, we introduce the model. In section 3, we estimate parameters for the model. In section 4, we briefly discuss the dynamics of the model and we determine numerically the nutrient removals at steady state operation. Finally, in section 5, we discuss our results.

2. MODEL DESCRIPTION

Let us consider a continuous reactor perfectly mixed (chemostat) of depth L, illuminated with an incident light intensity $I_{in}$, operated at a dilution rate $D$, and with a biomass $x(t)$ of microalgae (see Figure 1). The chemostat is fed with ammonium ($NH_4$), nitrate ($NO_3$), and phosphate ($PO_4$) at concentrations $s_{NH4,in}$, $s_{NO3,in}$, and $s_{PO4,in}$ respectively.

The evolution of $x$ can be derived from a mass balance, leading to the following equation:

$$\frac{dx}{dt} = [\mu(\cdot) - D - m]x,$$

(1)

where $\mu$ is the microalgae growth rate and $m$ is the mortality rate.

![Fig. 1. Scheme of a chemostat fed with wastewater.](image)

| Variable | Definition | Unit |
|----------|------------|------|
| $x$      | Algal biomass | mgCODL$^{-1}$ |
| $q_N$    | Nitrogen cell quota | mgN mgCOD$^{-1}$ |
| $q_P$    | Phosphorus cell quota| mgP mgCOD$^{-1}$ |
| $\theta$ | Chlorophyll quota | mgChl mgCOD$^{-1}$ |
| $s_{NH4}$ | NH$_4$ external concentration | mgN L$^{-1}$ |
| $s_{NO3}$ | NO$_3$ external concentration | mgN L$^{-1}$ |
| $s_{PO4}$ | PO$_4$ external concentration | mgP L$^{-1}$ |
| $x_N$    | Nitrogen biomass | mgN L$^{-1}$ |
| $x_P$    | Phosphorus biomass | mgP L$^{-1}$ |

Table 1. List of variables involved in the model.

![Fig. 2. Correlation between $\theta$ and $k$. Experimental data is taken from the work of Wagner et al. (2018). The linear regression is given by $k = 2.5459 \times \theta + 0.0484$.](image)

The growth rate $\mu(\cdot)$ depends on the quotas of nitrogen, $q_N$, and phosphorus, $q_P$, and on the light availability in the medium. This co-limitation has been described by the multiplicative model, the threshold model (law of minimum), or the combination of both models. Here, we follow the idea of Guest et al. (2013) i.e. nutrient co-limitation is described by the law of minimum (normalized as done by Bougaran et al. (2010)) and then multiplied by the growth rate under light limitation i.e.

$$\mu(\cdot) = \min \left\{ \frac{1 - \frac{q_N}{K_{q_N}}}{1 - \frac{q_P}{K_{q_P}}}, \frac{1 - \frac{q_N}{K_{q_N}}}{1 - \frac{q_P}{K_{q_P}}} \right\} \mu_I(\cdot),$$

(2)

with $q_{N0}$ and $q_{P0}$ the minimum quotas for life, $q_{NL}$ and $q_{PL}$ the values of nitrogen and phosphorus quota when the growth rate has reached its maximum value, and $\mu_I(\cdot)$ the growth rate when nutrient limitations are ignored. $\mu_I(\cdot)$ is given by:

$$\mu_I(\cdot) = \frac{1}{L} \int_0^L p(I(z), \theta) dz,$$

(3)

with $p$ the local specific growth rate of microalgae (without nutrient limitation), and $I(z)$ the light intensity at a distance $z$ from the illuminated surface. $I(z)$ is determined with the Lambert-Beer law:

$$I(z; I_{in}) = I_{in} e^{-(k_0 + K_{bg})z},$$

(4)

with $k$ and $K_{bg}$ light attenuation coefficients associated to microalgae and to the background medium respectively. The specific microalgae extinction coefficient depends on the chlorophyll quota $\theta$ (see Figure 2):

$$k = k_1 \theta + k_2,$$

(5)

with $k_1$ and $k_2$ non-negative coefficients. The function $p$ follows a Haldane form:

$$p(I, \theta) = \frac{p_{max}}{I + \frac{p_{max}}{cm(\theta)} \left( \frac{I}{I^*} - 1 \right)^2},$$

(6)

where $I^*$ and $p_{max}$ are the irradiance at which $p$ is maximal and the specific maximum growth rate respectively, while
\[ N\text{H}_4 \text{ uptake rate: } \rho_{N\text{H}_4} (\cdot) = \begin{cases} \frac{\rho_{\text{max},N\text{H}_4}}{K_{N\text{H}_4} + s_{N\text{H}_4}} & \text{if } q \leq q_{N\text{L}} \text{,} \\ 0 & \text{if } q > q_{N\text{L}}. \end{cases} \]

\[ \text{NO}_3 \text{ uptake rate: } \rho_{\text{NO}_3} (\cdot) = \begin{cases} \frac{\rho_{\text{max},\text{NO}_3}}{K_{\text{NO}_3} + s_{\text{NO}_3}} & \text{if } q \leq q_{\text{NL}} \text{,} \\ 0 & \text{if } q > q_{\text{NL}}. \end{cases} \]

\[ \text{PO}_4 \text{ uptake rate: } \rho_{\text{PO}_4} (\cdot) = \begin{cases} \frac{\rho_{\text{max},\text{PO}_4}}{K_{\text{PO}_4} + s_{\text{PO}_4}} & \text{if } q \leq q_{\text{PL}} \text{,} \\ 0 & \text{if } q > q_{\text{PL}}. \end{cases} \]

Table 2. Uptake rate functions. \( \rho_{\text{max},N\text{H}_4}, \rho_{\text{max},\text{NO}_3}, \text{ and } \rho_{\text{max},\text{PO}_4} \text{ are maximal uptake rates, } \) and \( K_{N\text{H}_4}, K_{\text{NO}_3}, \text{ and } K_{\text{PO}_4} \) are half-saturation constants. Note that \( K_{N\text{H}_4} \) also indicates the preference of algae for \( N\text{H}_4 \) over \( \text{NO}_3 \).

\( \alpha_0 \) is the initial slope of the light response curve for a chlorophyll quota \( \theta_0 \). The parameter \( \theta_0 \) must be chosen. We note that the initial slope of the function \( p \) normalized to chlorophyll \( p^\text{Chl}(I, \theta) := p(I, \theta)/\theta \), is \( \alpha_0/\theta_0 \) i.e. independent of \( \theta \). This is consistent with Bernard et al. (2015).

Based on the Droop model (see Droop (1968); Bougaran et al. (2010)) and on the model presented by Bernard et al. (2015) for photoacclimation, the evolution of the quotas of nitrogen, phosphorus, and chlorophyll is described by:

\[
\frac{dq_N}{dt} = \rho_{N\text{H}_4} (\cdot) + \rho_{\text{NO}_3} (\cdot) - \mu (\cdot) q_N,
\]

\[
\frac{dq_P}{dt} = \rho_{\text{PO}_4} (\cdot) - \mu (\cdot) q_P,
\]

\[
\frac{d\theta}{dt} = \mu (\cdot) [\theta^* (\cdot) - \theta],
\]

Here, \( \rho_{N\text{H}_4} (\cdot), \rho_{\text{NO}_3} (\cdot), \text{ and } \rho_{\text{PO}_4} (\cdot) \) represent the uptake rates of \( N\text{H}_4, \text{NO}_3, \text{ and } \text{PO}_4 \) respectively, and \( \theta^* (\cdot) \) represents the chlorophyll quota at which microalgae tend to adapt. As done by Mairiet et al. (2011), we assume that the chlorophyll acclimation rate is the growth rate. The uptake rate functions are given in Table 2. In line with Gotham and Rhee (1981), the uptake rate of a nutrient increases with the bulk concentration of the nutrient and decreases with the respective quota. Following the work of Bougaran et al. (2010), we consider that the uptake of \( N \) (in form of \( N\text{H}_4 \) or \( \text{NO}_3 \)) is enhanced by the phosphorus cell quota, and as reported by Dortch (1990), we consider that microalgae prefer \( N\text{H}_4 \) over \( \text{NO}_3 \). For \( \theta^* \), we assume that:

\[
\theta^* (\cdot) = \gamma_m q_N \frac{k_I}{I + k_I},
\]

with \( k_I \) and \( \gamma_m \) photoacclimation parameters, and \( I := \frac{1}{L} \int_0^L I(z)dz \) the average light intensity in the medium.

The evolution of the external concentrations of ammonium, nitrate, and phosphate is given by:

\[
\frac{ds_{N\text{H}_4}}{dt} = D(s_{N\text{H}_4,\text{in}} - s_{N\text{H}_4}) - \rho_{N\text{H}_4} (\cdot) x.
\]

\[
\frac{ds_{\text{NO}_3}}{dt} = D(s_{\text{NO}_3,\text{in}} - s_{\text{NO}_3}) - \rho_{\text{NO}_3} (\cdot) x.
\]

\[
\frac{ds_{\text{PO}_4}}{dt} = D(s_{\text{PO}_4,\text{in}} - s_{\text{PO}_4}) - \rho_{\text{PO}_4} (\cdot) x.
\]

A summary of all the state variables is given in Table 1.

3. PARAMETER ESTIMATION

We estimate the parameters of the model with experimental data from Wágner et al. (2018). Experiments were carried out in a 8-L batch reactor with a mixed green microalgal consortium consisting mainly of Chlorella sorokiniana and Scenedesmus sp.. Constant aeration with \( CO_2 \) enriched air (5% \( CO_2 \)) at a flow rate of 20 L/h was used to mix biomass and to provide \( CO_2 \). The reactor is illuminated from above (flat-surface) with an incident light intensity of 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The height of the culture (or light-path length) is 0.4 m. The measured variables are the microalgae concentration \( x \), the nitrogen biomass (\( x_N := x q_N \)), the phosphorus biomass (\( x_P := x q_P \)), the chlorophyll quota \( \theta \), and the external nutrient concentrations.

The quotas \( q_{N0} \) and \( q_{P0} \) are estimated as the minimum of the experimental values of the respective quota, and the quotas \( q_{N0} \) and \( q_{P0} \) are estimated as a 10% higher than the maximum value of the respective quota. The parameters \( \gamma_m, \alpha, \rho_{N\text{H}_4,\text{max}}, \rho_{\text{NO}_3,\text{max}}, \rho_{\text{PO}_4,\text{max}} \), and \( m \) are determined with the optimization solver of MATLAB \texttt{fminsolve}. The objective function to minimize is the square error normalized to the range of the observed data i.e.

\[
\sum_{Y=x_N, x_P, \ldots} \left( \frac{Y_{\text{model},i} - Y_{\text{obs},i}}{Y_{\text{obs},\text{max}} - Y_{\text{obs},\text{min}}} \right)^2
\]

The solver is initialized with parameters from literature (Wágner et al. (2016); Mairiet et al. (2011)). Not estimated parameters are taken from literature (see Table 3). Figure 3 represents the experimental data used for estimating the parameters and model simulation, showing the good fitting performances of the model. Further validation with other experimental data should deserve future work.
Fig. 3. Simulations of (1)-(7)-(9) with parameters from Table 3 and experimental data from Wágner et al. (2018).

**Table 3. List of parameters involved in the model.** *Mairet et al. (2011), **Huisman et al. (2002), ***Wágner et al. (2016). Parameters estimated with fminsearch in Matlab.

| Parameter | Value | Unit |
|-----------|-------|------|
| $\gamma_m$ | 0.01 | (Estimated) |
| $k_1$ | 97* | |
| $k_1$ | 2.54 | (Fig. 2) |
| $k_2$ | 0.0484 | (Fig. 2) |
| $K_{bg}$ | 7.2** | |
| $\theta_0$ | 0.01 | (Chosen) |
| $\alpha$ | 0.0108 | (Estimated) |
| $I^*$ | 758*** | |
| $p_{max}$ | 3.6*** | |
| $q_{NO}$ | 0.0264 | (Estimated) |
| $q_NL$ | 0.0833 | (Estimated) |
| $p_{NH_4, max}$ | 4.5 | (Estimated) |
| $p_{NO_3, max}$ | 0.61 | (Estimated) |
| $K_{NH_4}$ | 6.7*** | |
| $K_{NO_3}$ | 6.87*** | |
| $q_{P0}$ | 0.0027 | (Estimated) |
| $q_{PL}$ | 0.0058 | (Estimated) |
| $p_{PO_4, max}$ | 0.01 | (Estimated) |
| $K_{PO_4}$ | 4.5*** | |
| $m$ | 0.044 | (Estimated) |

4. REMOVAL CAPACITY OF THE SYSTEM

Simulations of the model for different initial conditions and operation parameters (depth, dilution rate, and incident light intensity) show that any trajectory approaches towards a steady state; either the washout characterized by the absence of microalgae, or a positive steady state characterized by the presence of microalgae. In some cases, the positive steady state is reached by any solution starting with a positive initial microalgae concentration. In other cases, especially when microalgae are highly photoinhibited (high incident light with low depth for example), the model faces bi-stability, i.e. depending on the initial
conditions, the trajectories approach to the washout or the positive steady state (see Figure 4). In the case of no limitation by nutrients, the existence of bi-stability is due to an Allee effect resulting from photo-inhibition (Gerla et al. (2011)). Alle effect is stronger for higher light intensities and lower depths (Martínez et al. (2018)). A complete analysis of model dynamics with nutrient limitation is difficult to achieve due to model complexity, but the same phenomenon seems to occur.

We define the steady state areal removal rate of the nutrient $Y$ ($Y = NH_4, NO_3, PO_4$) by:

$$ R_Y := DL(s_{Y,in} - s'_Y), $$

(11)

with $s'_Y$, the concentration of the nutrient $Y$ associated to the positive steady state. $R_Y$ indicates the quantity of nutrient $Y$ that is removed per day per unit of area of the reactor.

To evaluate the nutrient removal rate, we run the model for 300 days with high initial microalgae concentrations (300 gCOD/m$^3$) to avoid the extinction if bistability occurs. Thus, $s'_Y$ is given by the evaluation of the simulated $s_Y$ at the day 300. For the evaluations, we consider three different incident light intensities, 300 (no microalgae suffer from photo-inhibition), 1000, and 3000 µmol m$^{-2}$ s$^{-1}$ (microalgae closer to the surface suffer from photo-inhibition), we consider four different depths 0.1, 0.2, 0.4, and 0.6 m, and different dilution rates in the range of 0 to 1 d$^{-1}$. The results are shown in Figure 5.

Figure 5 shows that for an incident light intensity of 300 µmol m$^{-2}$ s$^{-1}$, the removal rates are better for a depth of 20 cm. However, for high incident light intensities, 1000 µmol m$^{-2}$ s$^{-1}$ and 3000 µmol m$^{-2}$ s$^{-1}$, there is a positive relation between nutrient removal rates and the HRAP depths. We also observe that similar nutrient removal rates are reached for incident light intensities of 1000 µmol m$^{-2}$ s$^{-1}$ and 3000 µmol m$^{-2}$ s$^{-1}$. While for 300 µmol m$^{-2}$ s$^{-1}$ the nutrient removal rate is approximately six times lower.

Figure 5 also shows that the range of dilution rates at which the HRAP removes nutrients (i.e. the system reaches a positive steady state) is highly dependent on the water depth and on the incident light. This range is the highest for an incident light of 1000 µmol m$^{-2}$ s$^{-1}$.

5. DISCUSSION AND CONCLUSIONS

We built a mathematical model for describing microalgae growth in HARPs under limitation by light, phosphorus, and nitrogen. The model shows a good performance in reproducing experimental data, describing accurately the consumption of the different nutrients in the medium.
With the help of numerical computations, we showed the impact of the depth, of the incident light intensity, and the dilution rate on the nutrient removal rates. The impact of one of these parameters strongly depends on the other two parameters. Our results are supported by some experimental studies. For example, Kim et al. (2018) has recently shown that in a HRAP illuminated with 100 \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \) a depth of 20 cm is better than depths of 30 and 40 cm. This is consistent with our results for the lowest incident light intensity. Arbib et al. (2017) showed experimentally that for an illumination of 250 \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \), under a 14 : 10 h light:dark cycle, a depth of 30 cm is more efficient than 15 cm. This is consistent with our results for high light intensities.

As a future work, a better characterization of the effect of chlorophyll content on microalgae growth will be carried out. Actually, experimental results of Neidhardt et al. (1998) and Anning et al. (2000) show that the maximum specific growth rate and the irradiance at which growth is maximal can vary with the chlorophyll content. This could strongly affect how microalgae are photo-limited or inhibited, and thus change system performances.

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