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# Dynamical Metabolic Model for Optimizing Biotin-Regulated Lipid Production in Microalgae-Bacteria Symbiosis

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**Abstract:** Exploiting natural symbioses to enhance productivity of bioprocesses is an emerging trend. For optimizing such complex associations of microorganisms, a model of symbiotic interactions is vital. This challenging task has attracted much attention. Here, a reduced metabolic model describing a symbiotic interaction between bacteria *E. coli*, overproducing vitamin biotin ($B7$), and microalgae *Chlorella* is developed. The symbiosis involves $B7$ exchange, impacting lipid synthesis regulation in microalgae. Our model shows a trade-off between light availability and biotin production, leading to an optimization problem for lipid production. We numerically determine the optimal conditions, demonstrating the feasibility of this strategy to enhance microalgae cultivation.

*Keywords:* Bioprocess control; Microbial technology; Microalgae; Optimization; Model reduction; Metabolic engineering

## 1. INTRODUCTION

Symbiotic interactions between microalgae and bacteria are gaining increasing attention due to their significant role in natural ecosystems (Ashraf et al., 2023). Once tamed, the complex relationships between algae and bacteria is seen as a way for improving algae based bioprocesses design and efficiency (Nagarajan et al., 2022). Growing bacteria together with microalgae is interesting, since some molecules necessary for algal growth can be produced by the bacteria. Essential vitamins are required by all living cells, but not all microalgae can synthesize them, making them auxotroph (Cooper et al., 2019). In some cases, the algae is not completely dependent on a vitamin, but an external supply can benefit biomass growth. This is the case of *C. reinhardtii* when it grows in the presence of an external supply of cobalamin (B12), which enables a B12-dependent pathway for the synthesis of methionine that has a higher reaction rate (Kazamia et al., 2012). The exogenous supply of vitamins from bacteria to microalgae plays a key role in designing more sustainable cultivation systems (Tandon et al., 2017) which will not need that addition of external expensive vitamins.

Modeling of algae-bacteria interactions can guide the development of more robust and environmentally friendly processes with reduced need for external nutrients or micronutrients. This model-based approach can lead to improvements in the design of the cultivation system and offers the possibility to set up optimal strategies to increase the overall efficiency of the process. A model can also be a useful tool to guide metabolic and genetic engineering. Advances in the modeling of these complex symbiotic interactions will help to decipher the dynamics of the ecosystem and eventually will pave the way for more efficient and sustainable processes.

Recent research has explored metabolic and genetic engineering approaches to enhance lipid production in microalgae. These efforts have primarily focused on overexpressing genes associated with lipid synthesis, such as genes associated with the enzyme acetyl-CoA carboxylase (ACCase) (Sun et al., 2019). Biotin (vitamin B7) is a necessary cofactor in lipid biosynthesis for all organisms because it acts together with ACCase to promote the transformation of acetyl-CoA into malonyl-CoA, which is the primary building block for the synthesis of fatty acids (Huerlimann and Heimann, 2013).

According to Croft et al. (2006) few microalgae species are biotin auxotrophs, and usually, they are also auxotrophs for thiamine or cobalamin. The necessity of biotin for lipid production is well established by our knowledge of metabolic pathways, but most works focused on genetic engineering trying to over synthesize the enzyme ACCase

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without considering the influence of biotin. The variety of contradictory results in the literature (Sun et al., 2019), regarding the effect of ACCase in lipid accumulation, demonstrates the complexity of the lipid synthesis pathway and its regulation. Also, little attention has been paid to the actual concentration of biotin, and its effect on lipid accumulation. Furthermore, biotin is a costly vitamin to produce and its biological synthesis has only recently been understood (Wei et al., 2021).

In this work, we consider this possible pathway of regulation of lipid production via biotin, by considering a co-culture of a biotin over-producer *E. coli* (Wei et al., 2021) and of *Chlorella*. We adapt the metabolic model from Pessi et al. (2023) by including the lipid content of *Chlorella* and by considering its dependency on the biotin availability. We assess, via simulations, whether the model supports our strategy at the steady-state and in dynamical conditions, considering diurnal changes in light intensity.

## 2. METHODS

### *2.1 Metabolic model*

Here, we consider a reactor containing the bacterial species *E. coli* ($E$) and the microalgal species *Chlorella* ($B$). The bacteria grow heterotrophically, consuming glucose ($GLC$) and producing vitamin $B7$, which catalyzes the production of lipids ($PA$) in the algae. While microalgae grows autotrophically, using the light energy. Fig. 1 presents a simplified scheme of the symbiotic model.

The proposed model is partially based on the metabolic model of *Chlorella* from Pessi et al. (2023). Here, we only consider the autotrophic growth of *Chlorella* and detail the lipid synthesis pathway. The model for *Chlorella* is derived from a metabolic network after a step of dynamical reduction, following the DRUM approach (Baroukh et al., 2014), which initially had 188 reactions and 173 metabolites.

Briefly, in the DRUM framework, a metabolic model with $n_r$ reactions and $n_m$ metabolites, represented by a stoichiometric matrix $S \in \mathbb{R}^{n_m X n_r}$ is reduced by dividing the metabolic network into subnetworks which are at steady state and are connected by metabolites that are allowed to accumulate. Therefore, the metabolites at steady state do not impact the dynamics of the system, and it is not necessary to represent them in the new reduced network. Macro reactions are derived from the subnetworks, representing the overall biochemical transformation taking place in the subnetwork. Here, we consider four subnetworks. Three subnetworks for *Chlorella* - Lipid Synthesis, Chloroplast and Biomass Synthesis - and one subnetwork for *E. coli*. The accumulation variables are glyceraldehyde 3-phosphate ($GAP$), and phosphatidic acid ($PA$), with $PA$ representing lipid accumulation in our metabolic model. Both are expressed in cellular concentration (grams per grams of biomass).

For *E. coli*, we consider a biotin over-producer as described in Wei et al. (2021) and an initial metabolic model containing 2251 reactions and 1136 metabolites (Orth et al., 2011). It is reasonable to assume for bacteria that all internal metabolites are at steady state, not requiring the creation of subnetworks. Therefore, the macroscopic reactions can be derived directly from the list of elementary flux modes of the system. Since, enumeration of the whole list of elementary flux modes is computationally very burdensome, we reduce the considered reactions by performing Flux Balance Analysis when the objective function is the biomass reaction and when it is the synthesis of $B7$. Following the selection of the elementary flux modes with the highest yield for the two cases, we end up with two macro reactions: one for biomass production and the other for the synthesis of $B7$.

The concentration dynamics for intracellular molecules within a metabolic network in a continuous stirred-tank reactor can be represented as follows:

$$\frac{dM}{dt} = Sv(M)B + (M_{in} - M)D \tag{1}$$

Here, $M \in \mathbb{R}^{n_m}$ represents a vector of the concentration of metabolites, $M_{in} \in \mathbb{R}^{n_m}$ is a vector of the concentrations at the inlet of the reactor, $S \in \mathbb{R}^{n_m X n_r}$ is the stoichiometric matrix, $v \in \mathbb{R}^{n_r}$ denotes the vector of reaction kinetics, $B$ is the functional biomass, and $D$ is the dilution rate (influent flow rate divided by the reactor volume). In the DRUM framework, total biomass, $X$, is the sum of $B$ and the accumulating metabolites. The concentration of the internal metabolites ($c$) can be represented as a fraction of the biomass, in this case the dynamical equation is:

$$\frac{dc}{dt} = Sv(M) - c\mu \tag{2}$$

where $\mu$ is the growth rate.

The dynamical metabolic model is formulated by the subsystem of ordinary differential equations described below, for a continuous reactor. The values and descriptions of the parameters are shown in Table 1.

$$\frac{dE}{dt} = \mu_{Emax}\frac{GLC}{GLC + K_E}E - (D + m_E)E \tag{3}$$

Here, $E$ is the *E. coli* biomass concentration, and $GLC$ denotes the glucose concentration. The equation represents heterotrophic growth of $E$, using glucose as a carbon substrate.

$$\begin{aligned}\frac{dGLC}{dt} &= -\gamma_E\mu_{Emax}\frac{GLC}{GLC+K_E}E \\ &+(GLC_{in} - GLC)D\end{aligned} \tag{4}$$

In the above equation, $GLC_{in}$ signifies the influent glucose concentration. $GLC$ is consumed only by *E. coli* and it is supplied in the influent.

$$\frac{dB7}{dt} = \mu_{B7_{max}}\frac{GLC}{GLC + K_{B7}}E - (m_{B7} + D)B7 \tag{5}$$

$B7$ is the biotin concentration in the reactor, produced by *E. coli* at a maximum rate of $\mu_{B7_{max}}$, following a Monod relation dependent on glucose concentration, with a decay rate of $m_{B7}$.

$$\begin{aligned}\frac{dGAP}{dt} &= \mu_{GAP}(I, X + E) + \mu_{PA^{-1}_{max}}PA - \gamma_{GAP}\mu_B \\ &-\mu_{PA_{max}}GAP\frac{B7/B}{B7/B+K_{PA}} - \mu_B GAP\end{aligned} \tag{6}$$

In Equation 6, $GAP$ denotes the cellular fraction of Glyceraldehyde 3-phosphate in *Chlorella*, $PA$ is the cellular fraction of lipids (Phosphatidic acid) in *Chlorella*, $B$ is the *Chlorella* functional biomass, $\mu_{GAP}$ denotes the rate of $GAP$ production via the photosynthetic pathway, $\mu_B$ is the growth rate of *Chlorella*. $GAP$ is utilized as a reactant

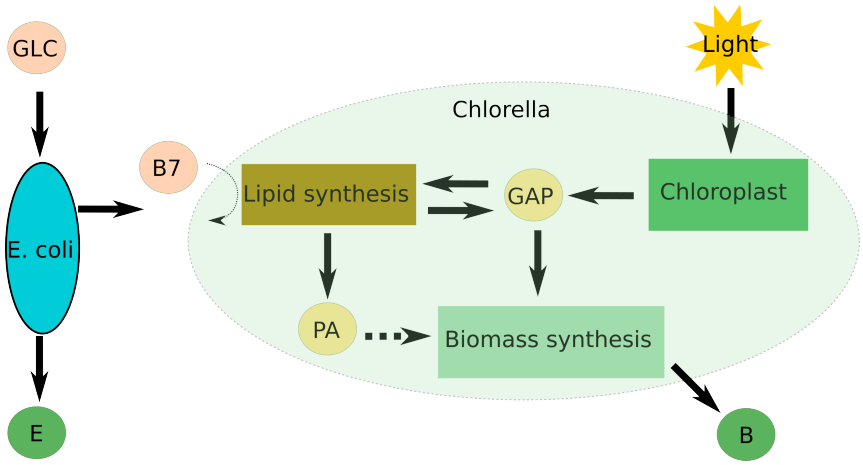


Fig. 1. A simplified representation of the metabolic network, showing the 6 state variables and reactions. $E$: bacteria *E. coli*, $B$: microalgae *Chlorella* functional biomass, $B7$: biotin, $GLC$: Glucose, $PA$: lipids, $GAP$: glyceraldehyde 3-phosphate.

for functional biomass production and lipid production, at a rate regulated by $B7$. Its concentration is diluted by the growth of *Chlorella*. Besides being produced by photosynthesis, $GAP$ can be produced via the reverse reaction of lipid synthesis.

$$\begin{aligned}\frac{dPA}{dt} &= \gamma_{PA_{GAP}}\mu_{PA_{max}}GAP\frac{B7/B}{B7/B+K_{PA}} \\ &-\gamma_{PA_{GAP}}\mu_{PA^{-1}_{max}}PA - \mu_B PA\end{aligned} \tag{7}$$

Lipids ($PA$) serve as a carbon reserve, produced by a reversible reaction from $GAP$, with consumption back to $GAP$ regulated by its own concentration.

$$\frac{dB}{dt} = (\mu_B - D)B \tag{8}$$

Here, $\mu_B = \mu_{B_{max}}GAP$, indicating the regulation of *Chlorella* biomass production rate by the internal concentration of $GAP$. The total (or dry weight) microalgal biomass, $X$, is calculated by the following equation:

$$X = \frac{B}{1 - PA - GAP} \tag{9}$$

The rate of synthesis of $GAP$ from photosynthesis, $\mu_{GAP(I,X+E)}$, is given by the average growth in the reactor, which depends on the light intensity reaching the reactor and turbidity effects because of the particles present in the medium (Béchet et al., 2015):

$$\mu_{GAP(I,X+E)} = \frac{\mu_{GAP_{max}}}{\sigma(X+E)L}ln(\frac{K_I + \sigma I_0}{K_I + \sigma I}) \tag{10}$$

Here, the light attenuation due to biomass absorption and scattering is given by the Beer-Lambert equation:

$$I = I_0 exp(-\sigma(X+E)L) \tag{11}$$

where $I_0$ denotes the light intensity at reactor surface, L is the depth of the reactor, and $\sigma$ is calculated as the average of E. *coli* and *Chlorella* extinction coefficients:

$$\sigma = \frac{aX^{1-b} + \sigma_E E}{E + X} \tag{12}$$

### *2.2 Parameter calibration*

Model parameters related to biotin production and decay, namely $\mu_{B7}$, $K_{B7}$ and $m_{B7}$, are calculated using data reported by Wei et al. (2021) for the growth of an *E. coli* biotin over-producer. That work presents the growth of *E. coli* together with glucose and biotin concentration over time. The parameters are determined by minimizing the error between the model predicted and experimentally measured biotin concentration in the medium over time, using the Differential Evolution optimization algorithm. This algorithm is chosen for its capacity for global optimization, and efficient utilization of multiple

| Parameter | Value | Unit | Description |
|---|---|---|---|
| $\mu_{Emax}$ | 10.6 | $d^{-1}$ | Bacteria maximum growth rate |
| $K_E$ | 0.04 | $g.L^{-1}$ | Half-saturation constant for glucose consumption |
| $m_E$ | 0.33 | $d^{-1}$ | Mortality rate of bacteria |
| $\gamma_E$ | 1.84 | $\frac{gGLC}{gE}$ | Yield of glucose to bacteria biomass |
| $\mu_{B7}$ | 0.05 | $d^{-1}$ | Maximum production rate of biotin (vitamin B7) |
| $K_{B7}$ | 3.8 | $g.L^{-1}$ | Half-saturation constant of biotin production |
| $m_{B7}$ | 0.38 | $d^{-1}$ | Decay rate of vitamin B7 |
| $\mu_{PAmax}$ | 23.3 | $d^{-1}$ | Maximum production rate of lipids |
| $K_{PA}$ | $10^{-5}$ | $\frac{gB7}{gB}$ | Half-saturation constant of lipid production |
| $\gamma_{PA_GAP}$ | 3.87 | $\frac{gPA}{gGAP}$ | Yield of lipid production from GAP |
| $\gamma_{PA_B}$ | 0.31 | $\frac{gPA}{gB}$ | Yield of lipids to B |
| $\mu_{PA^{-1}}$ | 2.75 | $d^{-1}$ | Maximum GAP production rate from stored lipids |
| $K_I$ | 182 | $\frac{\mu mol}{g \cdot s}$ | Light half-saturation constant |
| $\sigma_E$ | 285 | $m^2 g^{-1}$ | E. coli extinction coefficient |
| $a$ | 117.2 | - | Light extinction coefficient |
| $b$ | 0.2 | - | Light extinction power coefficient |
| $\mu_{GAP_{max}}$ | 1.15 | $d^{-1}$ | Maximum GAP synthesis rate from photosynthesis |
| $\gamma_{GAP}$ | 3.79 | $\frac{gGAP}{gB}$ | Yield of GAP to microalgae biomass |
| $\mu_{B_{max}}$ | 3.64 | $d^{-1}$ | Maximum growth rate of microalgae biomass |
| $L$ | 0.15 | $m$ | Depth of the reactor |

Table 1. Parameters calibrated for the model.

computer threads (Storn and Price, 1997). Fig. 2 shows the calibration results, by comparing the simulations to the experimental data.

The parameters related to lipid synthesis for *Chlorella* were adapted from Baroukh et al. (2014) to represent internal cellular concentrations. All other parameters are the same as considered in Pessi et al. (2023). The half-saturation constant of biotin regulating the synthesis of lipids, $K_{PA}$ is the only undetermined parameter of the model. We consider as a first guess the value of $10^{-5} gB7/gB$, based on the experimental conditions of Magdouli et al. (2020).

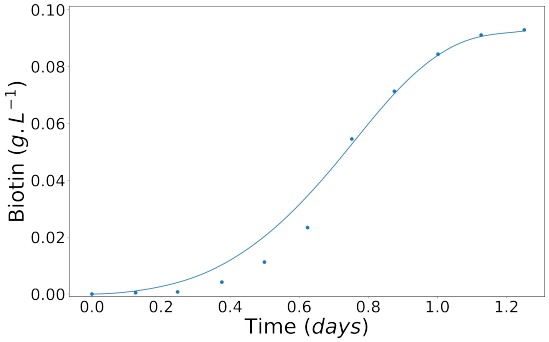


Fig. 2. Model calibration for biotin production. Experimental data points from Wei et al. (2021)

### *2.3 Steady-state optimization*

Here we analyze the lipid productivity ($P$) in the reactor outlet, considering constant light intensity ($I_0 = 300\ \mu mol/(m^2.s)$). The objective is to determine the optimal conditions for maximizing the lipid production rate at steady state, varying the dilution rate $D$ and the influent glucose concentration $GLC_{in}$:

$$\max_{D,GLC_{in}} : P(\cdot) = PA \cdot X \cdot D \cdot L \tag{13}$$

The values of $GLC$, $E$, $B7$, and $GAP$ at steady state can be directly computed from equations 3, 4, 5 and 8, respectively. The value of $B$ at steady state is determined numerically from the root of the equation 6, using Newton's algorithm. Finally, $PA$ is derived from the value of $B$ with Equation 7. The values of $D$ and $GLC_{in}$ maximizing $P$ at steady state are obtained using the Differential Evolution optimization algorithm (Storn and Price, 1997).

### *2.4 Dynamical optimization*

We consider the case of a day/night cycle, where the light fluctuates according to the following equation:

$$I_0(t) = max(300cos(2\pi t), 0) \tag{14}$$

The productivity equation must be integrated over the considered time period to deal with the system dynamics:

$$\max_{D,GLC_{in}} P_{dyn} := \int_0^{t_f} PA(t).X(t)D(t)Ldt \tag{15}$$

For the control variables ($u$), $D$ and $GLC_{in}$, we consider a suboptimal approximation of the optimal control problem. Drawing from insights obtained from previous optimized controlled microalgae systems (Martínez et al., 2022; De-Luca et al., 2019), and after preliminary tests using control vector parameterization, we adapt the shape of the optimal control in line with the pattern of light fluctuation, by the following piecewise control function:

$$u = \begin{cases} u_{max}|cos(2\pi(t^* - \tau)/T)| & ;\tau - T/4 < t^* < \tau + T/4 \\ 0 & ;\text{otherwise} \end{cases} \tag{16}$$

where $t^* = 24t\ mod\ 24$. The parameters $u_{max}, \tau$ and $T$ are calculated for each control variable, setting $P_{dyn}$ as the objective function, with a time range ($t_f$) of 100 days.

## 3. RESULTS AND DISCUSSION

### *3.1 Model dynamical behavior*

Fig. 3 shows the dynamical behavior at two different values of $GLC_{in}$, at constant light intensity of 300 $\mu mol/(m^2 s)$. The value of $D$ is chosen to be close to the optimum (see Fig. 4). It shows that the concentrations of *E. coli* and biotin rapidly reach their steady state, due to the faster heterotrophic growth rate. The response of *Chlorella* is different, due to the slower growth in autotrophic conditions. Several days are needed for the microalgal biomass to reach its steady state. The lipid content is dependent on the light availability, since lipids constitute a way to store carbon. Under normal day-night conditions, microalgae store carbon during the day in the form of lipids, and later consume them during the night.

For a low glucose concentration ($0.1 g/L$), *Chlorella* biomass increases over time, reducing light availability, and as a consequence, the lipid content decreases over time. When $GLC_{in}$ equals 0.5 $g/L$, a higher concentration of *E. coli* is reached at the beginning of the cultivation and, as a consequence, reducing the light transmitted to the culture. The microalgal biomass concentration thus decreases over time. It is important to note also, that since we consider an equilibrium between external and internal concentration of biotin, as the biomass decreases the internal biotin is higher ($B7/B$), and thus the internal lipids per biomass unit, $PA$, increases. This factor aggregates with the dynamics of light availability, since internal concentration of biotin will also determine the content of lipids at steady state.

This analysis highlights one of the most important behaviors of the model, the trade-off between light availability and biotin production. A higher glucose influent concentration increases the concentration of bacteria which reduces the transmitted light, and thus the average light intensity. Because of this behavior, as the production of biotin increases, there is eventually a loss of productivity due to reduced light availability. This is an important limitation of the co-culture when considering the autotrophic growth of microalgae. Given the probable range of $K_{PA}$, a concentration of *E. coli* moderately impacting light availability could be reached, while supplying the minimum required biotin to enhance lipid accumulation. The trade-off between light and biotin is also highlighted in Fig. 4. It shows how the lipid productivity changes with $D$ and $GLC_{in}$. Furthermore, it demonstrates the existence of a wash-out line, where for a fixed $GLC_{in}$ an increase in $D$ results in an abrupt loss of productivity.

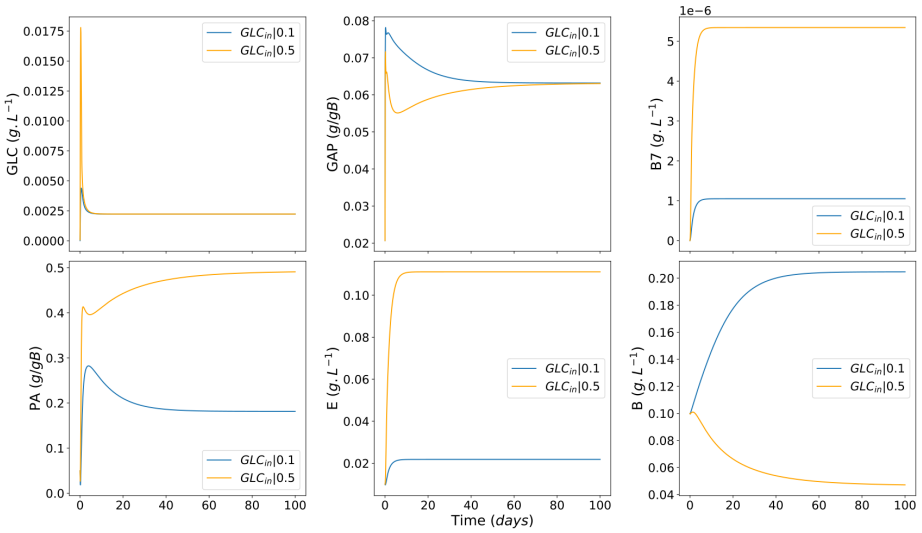


Fig. 3. Dynamics of the system at constant dilution rate ($D = 0.23d^{-1}$) and light intensity ($I_0 = 300\mu mol/(m^2 s)$), for two different influent concentrations of glucose. $GLC_{in}$ : $0.1g/L$ (blue) and $0.5g/L$ (orange).

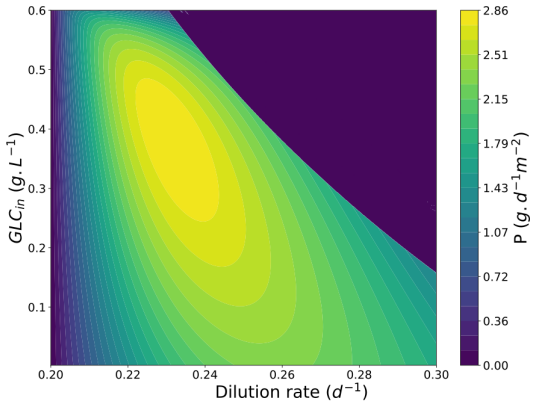


Fig. 4. Contour lines showing how lipid productivity at the steady state (P) changes as a function of the dilution rate (D) and the concentration of influent glucose ($GLC_{in}$).

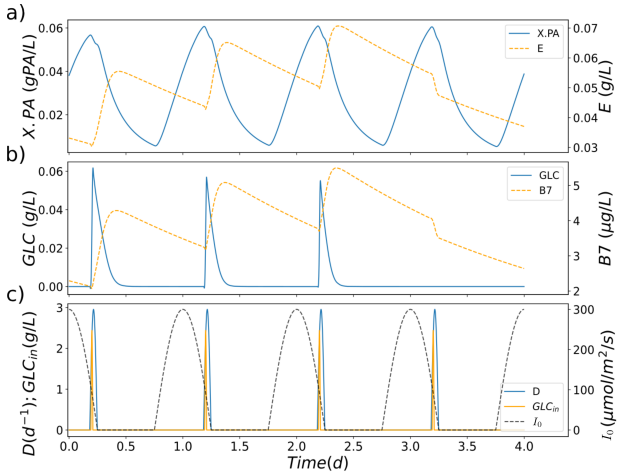


Fig. 5. Response of the system under fluctuating light, considering a suboptimal control strategy. a - Total lipid concentration ($X \cdot PA$) and bacterial concentration ($E$). b - Glucose ($GLC$) and biotin ($B7$) concentrations. c - Control variables: dilution rate ($D$) and influent glucose ($GLC_{in}$). Light intensity at the surface of the reactor ($I_0$).

### *3.2 Day/night cycle optimization*

Fig. 5 illustrates the response of the system to the suboptimal control strategy under fluctuating light conditions. The calibrated strategy consists in harvesting the system near the peak of total lipid concentration ($X.PA$), towards the end of the light cycle, allowing biomass replenishment during the first half of the light cycle. As growth does not occur during the night, harvesting only happens during daylight. Interestingly, although microalgal concentration appears to follow a 24h-cycle, the bacterial concentration progressively increases over the days, until it reaches a threshold concentration. At day 3, this threshold concentration leads to the absence of glucose in the medium, causing a rapid decline in the *E. coli* population. It is important to notice, that even under constant dilution rate and light intensity, because of the interactions between species, complex behaviors can emerge, such as the existence of limit cycles (Martínez et al., 2022).

### *3.3 Model limitations and perspectives*

The main uncertainty of the model is the relationship between the lipid content and the internal concentration of biotin. According to our model, the content of lipids at steady state is regulated by the internal concentration of biotin. We did not represent the internal production of biotin by *Chlorella*. It is reasonable to assume that the production of biotin by *Chlorella* is negligible compared to the quantity produced by the *E. coli* mutant. Also, it would be important to determine experimentally if the external biotin will down regulate the production of internal biotin by *Chlorella*. As seen in Kazamia et al. (2012), the gene expression of a vitamin B12 optional *C. reinhardtii* was regulated in the presence of a B12-

producing bacteria. Since biotin synthesis is costly, we could hypothesize a secondary effect where the growth of *Chlorella* would improve, which is not considered in the current state of the model.

Lipid accumulation is linked with limitations in the supply of nitrogen, which is not described in the kinetics of the model. Since there could be upregulation of ACCase in *Chlorella* in nitrogen deplete conditions, it is likely that supplementation of biotin will have a greater effect on the accumulation of lipids (Giridhar Babu et al., 2017). Adding vitamins enhanced lipid accumulation, but to different extents depending on if the culture is nitrogen depleted or replete (Fazeli Danesh et al., 2018).

## 4. CONCLUSIONS

This metabolic model is the first, to our knowledge, to include the dynamical influence of biotin in the accumulation of lipids. Additional experiments are now necessary to further validate the model and adapt it to the case of nitrogen limitation. It demonstrates the usefulness of the DRUM framework in modeling the dynamics of complex metabolic interactions, even in the case of a multi-species culture. Here we considered the interaction between two organisms through a particular vitamin, but in nature, this interaction is due to a large palette of chemical compounds. Representing such interactions, which are for most of them still unknown, will be a difficult challenge in the future. Being able to correctly model the metabolic interactions between bacteria and microalgae will make possible not only the optimization of current processes but also open new possibilities and new designs of bioprocesses.

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