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# Dynamics and control of a periodically forced microalgae culture <sup>★</sup>

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**Abstract:** Microalgae cultivation with wastewater is a promising way of reducing the energetic needs for wastewater treatment and the costs of biofuel production. However, the very turbid medium is not favorable for the development of microalgae. Indeed, light, the key element for photosynthesis, rapidly vanishes along depth due to absorption and scattering. Therefore it is crucial to understand the effects of the depth on turbid cultures. In this work, we study theoretically the long-term behavior of a continuous culture of microalgae exposed to a periodic source of light. By allowing periodic variations of the depth and the hydraulic retention time, we show that the microalgae population is forced to a periodic regime. Finally, we address numerically the problem of determining the optimal variations of the depth and the hydraulic retention time for maximizing the productivity of the culture in the periodic regime.

*Keywords:* Dynamics and control; Industrial biotechnology; Wastewater; Biomass productivity; Microalgae

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## 1. INTRODUCTION

Microalgae grown as a by-product of wastewater treatment in high rate algal ponds (HRAPs) represents a great opportunity for reducing costs of biofuel production (Park et al. (2011); Christenson and Sims (2011)). Moreover, HRAPs show a great potential for nutrient removal in wastewater treatment (Abdel-Raouf et al. (2012)). One problem with the cultivation of algae in wastewater 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 and the high content of particulate matter and colored substances (Morel and Bricaud (1981); Borowitzka (1998)). We refer to the light extinction due to all non-microalgae components as background turbidity. In the standard analyses of microalgae cultures, background turbidity is neglected. However, Martínez et al. (2018) showed theoretically that background turbidity results in a reduction of the productivity when increasing the depth of the system. Indeed, according to Larsdotter (2006), depths of the HRAP between 15 and

50 *cm* are generally recommended.

The objective of this paper is to give some insights in the optimization of biomass productivity by playing with the depth of the culture and considering a natural light source. In our approach, we combine the modelling approaches of Droop (1968) and Huisman et al. (2002) to build a model accounting for light and substrate limitation. Our model is periodically forced by the incident light with period of one day. We argue that by providing periodic input and output flows to the system, any solution of the model reaches a 1-day periodic regime i.e. approaches asymptotically a periodic solution. Thus, we study the productivity of the system in a periodic regime by controlling the input and output flow rates, or equivalently, the depth of the system and the hydraulic retention time.

This article is organized as follows. In section 2, we introduce the model of a microalgae culture given by a system of nonautonomous differential equations. In section 3, we study the dynamics of the model. The main result of this section (Theorem 2) gives sufficient conditions to ensure that any solution approaches asymptotically to a periodic solution characterized by the presence of microalgae (i.e. different from the washout). This result follows from reducing the model to a cooperative system and following some ideas of Smith (1997). In section 4, we state an optimal control problem for maximizing the microalgae productivity per day in the periodic regime. Periodic optimal

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problems have already been studied by Groganard et al. (2014); Bayen et al. (2015). Here, we present some numerical solutions obtained with the software BOCOP. Finally, we illustrate the asymptotic behavior of the model when repeating each day the 1-day periodic optimal control.

## 2. MODEL DESCRIPTION

Let us consider a perfectly mixed microalgae culture of depth  $L$  illuminated from above with an incident light  $I_{in}$ . We assume that light is attenuated exponentially according to the Lambert-Beer law i.e. at a distance  $z \in [0, L]$  from the illuminated surface, the corresponding light intensity  $I(x, z)$  satisfies

$$I(x, z) = I_{in}e^{-(kx+K_{bg})z}, \quad (1)$$

with  $x$  the microalgae concentration,  $k > 0$  the specific light attenuation coefficient of microalgae, and  $K_{bg} \geq 0$  the background turbidity. We assume that microalgae growth is limited by light and a substrate  $s$ . Based on the model of Droop (1968), we assume that the specific growth rate  $\mu$  depends on an internal cell quota  $q$ . The latter corresponds to an internal pool of nutrient per unit of biomass. The light intensity effects are described by a Monod type function. Thus, the specific growth rate is taken to be

$$\mu(I, q) = \mu_{max} \left(1 - \frac{q_0}{q}\right) \frac{I}{K_I + I} - r, \quad (2)$$

where  $r > 0$  is the respiration rate,  $q_0 > 0$  represents the value of  $q$  at which growth ceases,  $K_I > 0$  is a half-saturation constant, and  $\mu_{max} > 0$  is the maximal specific growth rate. Following Huisman et al. (2002), we compute the average growth rate in the reactor, denoted  $\bar{\mu}$ , by integrating the local growth rates over all the reactor

$$\bar{\mu}(\cdot) := \frac{1}{L} \int_0^L \mu(I(z), q) dz. \quad (3)$$

A simple computation leads to

$$\bar{\mu}(\cdot) := \frac{\mu_{max}}{(kx + K_{bg})L} \ln \left( \frac{K_I + I_{in}}{K_I + I_{out}(x)} \right) \left(1 - \frac{q_0}{q}\right) - r, \quad (4)$$

with  $I_{out}(x) = I(x, L)$  the light intensity at the bottom of the culture.

Cell quota  $q$  decreases with cell growth and increases with nutrient uptake, whose rate function is taken to be

$$\rho(q, s) = \begin{cases} \rho_{max} \frac{s}{K_s + s} d(q) & \text{if } q \leq q_L, \\ 0 & \text{if } q > q_L, \end{cases} \quad (5)$$

where  $\rho_{max}$  is the maximal uptake rate of nitrogen,  $q_L$  is the hypothetical maximal quota, and  $K_s$  is a half-saturation constant.  $d(q) \in [0, 1]$  is a down regulation term. While  $d(q)$  is usually taken as a linear function (Bougaran et al. (2010); Morel (1987)), here we take  $d(q)$  as

$$d(q) = \left( \frac{q_L - q}{q_L - q_0} \right)^2 \left( \frac{q_L - q}{q_L - q_0} + 3 \frac{q - q_0}{q_L - q_0} \right) \quad (6)$$

We note that  $d$  is the unique cubic function satisfying

$$d(q_0) = 1, d(q_L) = 0, \text{ and } d'(q_0) = d'(q_L) = 0. \quad (7)$$

The volume ( $V$ ) of the culture varies with the supply flow rate ( $Q_{in}$ ) and the withdrawal rate ( $Q_{out}$ ) according to

$$\dot{V} = Q_{in} - Q_{out}. \quad (8)$$

The transversal area ( $A$ ) of the culture is constant in time and along the depth of the culture. After dividing both sides in Eq.(8) by  $A$ , we obtain the following equation for the depth of the culture

$$\dot{L} = F_{in} - F_{out}, \quad (9)$$

with  $F_{in} := Q_{in}/A$  and  $F_{out} := Q_{out}/A$  linear flows. Mass balances in the culture give the following equations (see Chapter 2 in Dochain (2013) and Bougaran et al. (2010))

$$\begin{aligned} \dot{x} &= [\bar{\mu}(t, x, q, L) - \frac{F_{in}}{L}]x \\ \dot{q} &= \rho(q, s) - \bar{\mu}(t, x, q, L)q \\ \dot{s} &= \frac{F_{in}}{L}(s_{in} - s) - \rho(q, s)x, \end{aligned} \quad (10)$$

where  $s_{in}$  is the (constant) substrate influent concentration.

## 3. PERIODIC SYSTEM AND ASYMPTOTIC BEHAVIOR.

We assume that the incident light is variable in time according to

$$I_{in}(t) = I_{max} \max\{0, \sin(2\pi t/\omega)\}^2, \quad (11)$$

with  $\omega > 0$  the length of a day. Thus, by assuming that  $F_{in}$  and  $F_{out}$  vary periodically with a period  $\omega$ , the system (9)-(10) becomes an  $\omega$ -periodic system.

We use the following notation for the time-average of an  $\omega$ -periodic function <sup>4</sup>

$$\langle f \rangle := \frac{1}{\omega} \int_0^\omega f(t) dt. \quad (12)$$

By integrating Eq.(9) we obtain that

$$L(t) = L(0) + \int_0^t [F_{in}(s) - F_{out}(s)] ds. \quad (13)$$

We assume that

$$\langle F_{in}(t) - F_{out}(t) \rangle = 0.$$

Then  $L$  is an  $\omega$ -periodic function.

We define the total amount of limiting element both in the substrate and in the biomass by means of

$$S = s + xq. \quad (14)$$

<sup>4</sup> A function  $f$  is  $\omega$ -periodic if  $f(t + \omega) = f(t)$  for all  $t$

A simple calculation shows that  $S$  satisfies the differential equation

$$\dot{S} = D(t)(s_{in} - S), \quad (15)$$

with  $D(t) : \frac{F_{in}(t)}{L(t)}$  known as the dilution rate. We can rapidly verify that  $S(t) \rightarrow s_{in}$  as  $t \rightarrow \infty$  for any value of  $S(0)$ . Thus, the solutions of Eq.(10) approach the surface

$$s_{in} = s + xq.$$

We take advantage of this by dropping the equation for  $s$  and replacing  $s$  in Eq.(10) by  $s = s_{in} - xq$  in the second equation. This results in the two-dimensional system

$$\begin{aligned} \dot{x} &= [\bar{\mu}(t, x, q) - D(t)]x \\ \dot{q} &= \rho(q, s_{in} - xq) - \bar{\mu}(t, x, q)q \end{aligned} \quad (16)$$

In the following we study the asymptotic behavior of Eq.(16). Care must be exercised in extrapolating results obtained from the analysis of Eq.(16) to analogous results for Eq.(9)-(10). A discussion about this can be found in Smith (1997).

As  $s$  must be non-negative, initial conditions for Eq. (16) must satisfy  $x(0)q(0) \leq s_{in}$ . We note that if  $x(0) = 0$  (resp.  $x(0) > 0$ ), then  $x(t) = 0$  (resp.  $x(t) > 0$ ) for all  $t \geq 0$  (see Zanolin (1992) to find rigorous arguments). We also note that  $q(0) \geq q_0$  implies  $q(t) \geq q_0$  for all  $t \geq 0$ . Therefore we are only interested in solutions starting on the set

$$P := \{(x, q) ; x > 0, q \geq q_0, \text{ and } xq \leq s_{in}\}. \quad (17)$$

A solution  $(x, q)$  of Eq.(16) will be called an  $\omega$ -periodic solution provided each component is  $\omega$ -periodic. There is a trivial periodic solution corresponding to the absence of microalgae in the chemostat. Putting  $x = 0$  in the second equation of (16) results in

$$\dot{q} = \rho(q, s_{in}) - \bar{\mu}(t, 0, q)q. \quad (18)$$

The following proposition guarantees the uniqueness of the washout.

*Proposition 1.* Eq. (18) has a unique periodic solution  $q^*$  to which all solutions are attracted.

**Proof.** Since  $\dot{q}$  is strictly decreasing with  $q$ , we can use the same arguments as in the proof of Proposition 1.1 in Smith (1997).  $\square$

Thus, the trivial periodic solution is given by

$$x = 0, \quad q = q^* \quad (19)$$

The change of variables  $x_s := qx$  leads the system (16) to

$$\begin{aligned} \dot{x} &= [\bar{\mu}(t, x, x_s/x) - D(t)]x \\ \dot{x}_s &= \rho(x_s/x, s_{in} - x_s)x - D(t)x_s. \end{aligned} \quad (20)$$

Eq. (20) is a cooperative system i.e.

$$\frac{\partial \dot{x}}{\partial x_s}, \frac{\partial \dot{x}_s}{\partial x} \geq 0.$$

The importance of this system lies on the fact that any bounded solution of a planar, cooperative, periodic system of differential equations is asymptotic to a periodic solution ( see Chapter 7 in Smith and Waltman (1995)).

Since  $x_s$  satisfies the differential equation

$$\frac{dx_s}{dt} = x_s \left( \frac{\rho(q, s_{in} - x_s)}{q} - D \right), \quad (21)$$

we have that  $x_s(t) = x(t)q(t) \leq s_{in}$  for all  $t \geq 0$ . Consequently,  $P$  is positively invariant with respect to Eq. (16). Thus, if  $x(0) > 0$ , then  $x(t) \leq s_{in}/q_0$  for all  $t \geq 0$ . This implies that the set  $K := \{(x, x_s) ; x \in (0, \infty), q_0 < x_s/x, x_s \leq s_{in}\}$  is positively invariant with respect to (20). Consequently, any solution of (20) starting on  $K$  is bounded. Thus, any solution of Eq.(20) starting on  $K$ , or any solution of Eq. (16) starting on  $P$ , approaches a periodic solution. The following theorem gives conditions to avoid the washout  $(0, q^*)$  as an attracting periodic solution.

*Theorem 2.* If  $\langle \bar{\mu}(t, 0, q^*(t)) - D(t) \rangle$  is greater than 0, then any solution of Eq. (16) starting in  $P$  is asymptotic to an  $\omega$ -periodic solution of Eq.(16) with positive  $x$ -component.

**Proof.** We use the Floquet theory (see Chapter 7 in the book of Sideris (2013)) to determine the stability of the washout. The characteristic multipliers of the washout  $(0, q^*)$  are given by  $\rho_1 = \exp(\omega \langle a(t) \rangle)$  and  $\rho_2 = \exp(-\omega \langle c(t) \rangle)$  with

$$a(t) = \bar{\mu}(t, 0, q^*(t)) - D(t), \quad (22)$$

and

$$\begin{aligned} c(t) &= -\frac{\partial \rho}{\partial q}(q^*(t), s_{in}) + \bar{\mu}(t, 0, q^*(t)) \\ &+ q^*(t) + \frac{\partial \bar{\mu}}{\partial q}(t, 0, q^*(t)) > 0. \end{aligned} \quad (23)$$

Thus,  $\rho_1 > 1$  and  $\rho_2 < 1$ , and the washout is not stable. This implies that not all the solutions of Eq.(16) starting on  $P$  are asymptotic to the washout. Since any solution converges to an  $\omega$ -periodic solution, there exists an  $\omega$ -periodic solution different from the washout that attracts some solutions. Following the same arguments as in the proof of Proposition 1.3 in Smith (1997), we can conclude that this is true for any solution starting on  $P$ .  $\square$

Note that Theorem 2 does not say anything about the number of periodic solutions with positive  $x$ -component.

#### 4. OPTIMAL CONTROL PROBLEM

We define the *areal biomass harvest* on the interval of time  $[t_i, t_f]$  as

$$H = \int_{t_i}^{t_f} F_{out}(t)x(t)dt. \quad (24)$$

$H$  represents the quantity of biomass that is harvested on the interval of time  $[t_i, t_f]$  per unit of area.

Here we consider the case where the chemostat is operated in long term, with a periodic daily biomass production from the reactor outlet. The problem that we consider is the maximization of the biomass production over a single

day. The control variables are  $F_{in}$  ( $u_1$ ) and  $F_{out}$  ( $u_2$ ). The problem can be stated as

$$\begin{aligned} \max H(u_1, u_2) &= \int_0^\omega u_2(t)x(t)dt, \\ \text{s.t. } \dot{x} &= [\bar{\mu}(t, x, q, L) - u_1/L]x, \\ \dot{q} &= \rho(q, s_{in} - xq) - \bar{\mu}(t, x, q, L)x, \\ \dot{L} &= u_1 - u_2 \\ x(0) &= x(\omega) > 0, q(0) = q(\omega), L(0) = L(\omega) \\ u_1(t) &\in [0, F_{in,max}], u_2(t) \in [0, F_{out,max}] \end{aligned} \quad (25)$$

In the formulation of our problem we use Eq. (16) together with Eq. (9).

#### 4.1 Numerical results

We solve numerically the problem (25), with a direct method implemented in the software BOCOP (Bonnans et al. (2011)) (version 2.10). The problem is discretized by a two-stage Gauss-Legendre method of order 4 with 200 time steps. We consider a constant initialization, and the tolerance for IPOPT NLP solver is set at  $10^{-12}$ .

We consider two microalgae cultures; *culture 0* without background turbidity i.e.  $K_{bg} = 0$ , and *culture 1* with a background turbidity  $K_{bg} = 10 m^{-1}$ . For both cultures the rest of parameters are given in Table 1. The value of  $K_{bg}$  for the culture 1 was set as high as the numerical method allowed. We were not able to obtain convergence for values of  $K_{bg}$  equal to or higher than  $11 m^{-1}$ . Huisman et al. (2002) reported a background turbidity of  $7.2 m^{-1}$  for a nutrient-rich medium used in a laboratory culture.

Figures 1 and 2 show the results. In both cultures, algae concentration increases during half or almost all the day (first half of the period), then decreases at night (see Figure 1A). We note that in culture 1 the microalgae concentration is higher, and consequently the nutrient in the medium is lower (see Figure 1C). However, the daily microalgae productivity is  $1.7 gC m^{-2}$  in contrast to the culture 0 that reaches a daily microalgae productivity of  $4.0 gC m^{-2}$  (i.e.  $8.0 gDW m^{-2}$ ).

The control structures are Bang-Singular ( $u_1$ ) and Bang-Bang ( $u_2$ ) for both cultures (see Figure 2A). These controls result in a different depth for each culture. Figure 2B shows that there is a difference of almost  $40 cm$  during all the period of 24 hours with a lower depth for the culture that accounts for background turbidity.

Consider the controls ( $u_1, u_2$ ) (see Fig. 2A) obtained from the optimization of one periodic day-night cycle. We apply the respective control to each culture (i.e. to solve Eq.(9)-(16)) repeatedly for a long interval of time. The initial depth for each culture is equal to the initial depth of the respective optimal solution (see Figure 2B). The initial microalgae concentration and the initial quota are  $5 gC/m^3$  and  $0.28 gN/gC$  respectively for both cultures. Figures 3 and 4 show that the microalgae concentration approaches the optimal periodic microalgae concentration (Fig. 1A) found previously. For the culture 1 the microalgae concentration approaches more slowly to the periodic solution. Indeed, it needs 50 days in contrast to 30 days for the

Table 1. Parameters

Parameter	Value	Unit	Reference
$\mu_{max}$	1.72	$d^{-1}$	Huisman et al. (2002)
$K_I$	107	$\mu mol m^{-2} s^{-1}$	Huisman et al. (2002)
$k$	0.2	$m^2 gC^{-1}$	Béchet et al. (2013)
$\rho_{max}$	11.2	$gN gC^{-1} d^{-1}$	Bougaran et al. (2010)
$q_L$	0.28	$gN gC^{-1}$	Bougaran et al. (2010)
$q_0$	0.07	$gN gC^{-1}$	Bougaran et al. (2010)
$K_s$	0.0007	$gN m^{-3}$	Bougaran et al. (2010)
$r$	0.1	$d^{-1}$	-
$s_{in}$	10	$gN m^{-3}$	-
$I_{max}$	2000	$\mu mol m^{-2} s^{-1}$	-
$K_{bg}$	0 or 10	$m^{-1}$	-
$F_{in,max}$	1000	$L m^{-2} d^{-1}$	-
$F_{out,max}$	1000	$L m^{-2} d^{-1}$	-

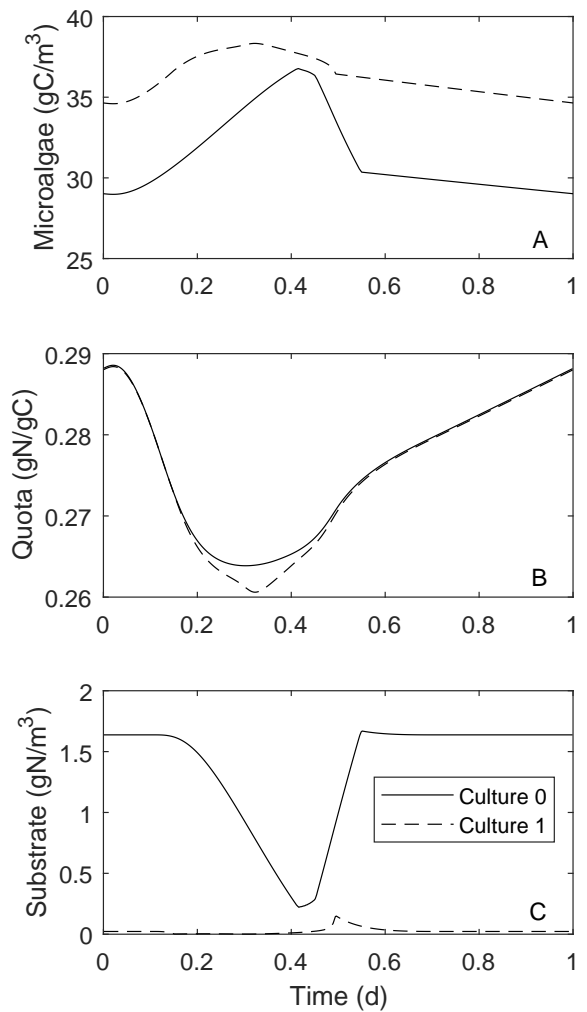


Fig. 1. Optimal solutions for problem (25) with two different background turbidities. A. Microalgae concentration. B. Cell quota. C. Substrate concentration in the medium.

culture 0.

By using the solver *bvp4c* (Shampine et al. (2000)) in Matlab we can determine numerically  $q^*$  and evaluate the

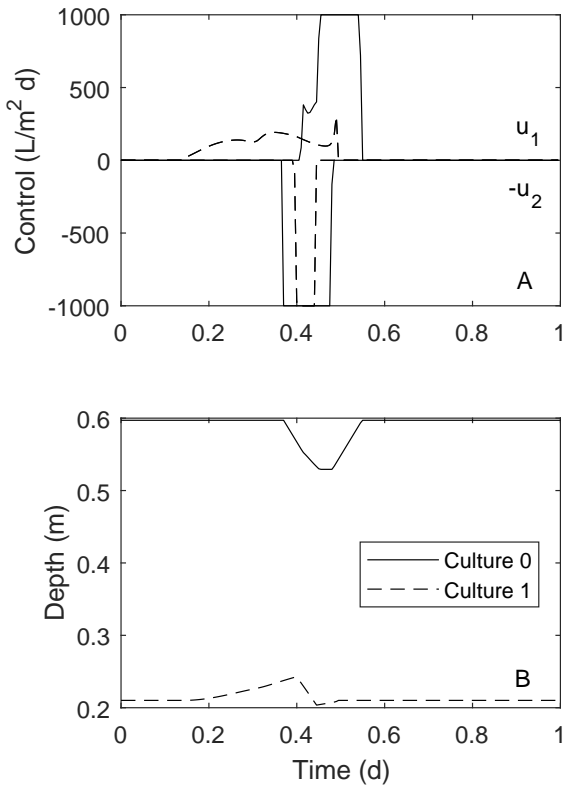


Fig. 2. Optimal solutions for problem (25) with two different background turbidities. A. Control variables. B. Depth of the culture.

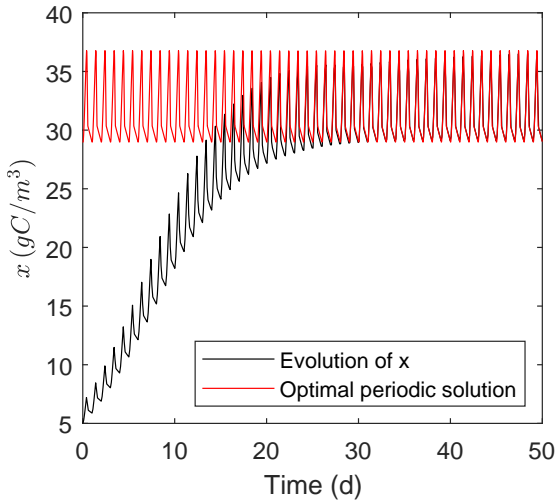


Fig. 3. Comparison of the optimal 1-day periodic microalgae concentration and the evolution of the microalgae with a low initial concentration when the periodic 1-day optimal periodic control is applied. Culture without background turbidity.

sufficient condition given in Theorem 2. For both cases, we have that  $\langle \bar{\mu}(0, q^*(t), t) - D(t) \rangle \geq 0.3$ , thus, any solution to Eq. (16) starting in  $P$  should converge to an  $\omega$ -periodic solution of the system with a positive microalgae concentration.

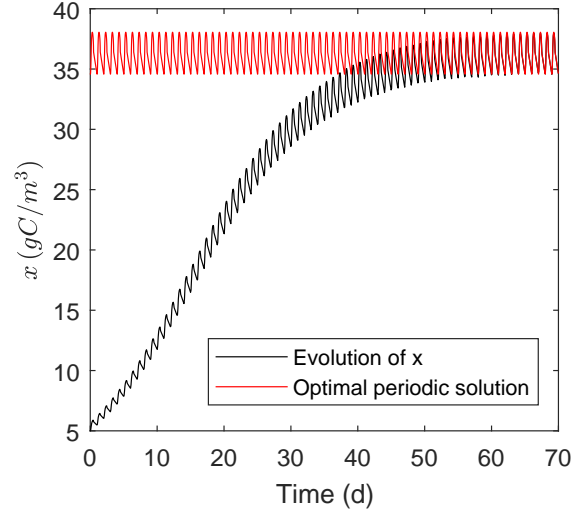


Fig. 4. Comparison of the optimal periodic microalgae concentration and the evolution of the microalgae with a low initial concentration when the periodic optimal turbidity is applied all the time. With background turbidity.

## 5. CONCLUSIONS AND FUTURE WORK

A first series of numerical simulations using a direct method illustrates the impact of the background turbidity on the choice of the depth of the culture for maximizing the microalgae productivity. We have shown that a background turbidity of  $10 m^{-1}$  can result in a reduction of  $40 cm$  of the optimal depth and a productivity loss of 55%. Thus, the background turbidity should not be neglected. The choice of the depth is not trivial and should be adapted to the overall turbidity, in particular when both light and nutrient limitations can occur. This choice should also include thermal considerations, since low depth induces lower thermal inertia. Processes with low depth are likely to overwarm very rapidly in hot seasons (DeLuca et al. (2017)) and a trade-off between light and temperature performances should be found.

We also studied theoretically the long-term behavior of the microalgae population when the system is operated periodically. We established sufficient conditions to ensure that any solution of the model, independently of the initial conditions, is attracted to an oscillatory solution with a positive microalgae concentration.

Photoinhibition (i.e. reduction of the photosynthetic rate due to high light intensities) should be included in a future work. Low depths of the culture could enhance the photoinhibitory process resulting in a lower productivity, and for low initial microalgae concentrations the population could go extinct (see Gerla et al. (2011); Martínez et al. (2017)).

In this work, we emphasized the productivity of microalgae using wastewater streams without paying attention to the aspect of wastewater treatment. In a future work, other

optimization problems will be studied. For example, the maximization of the removal of nitrogen with restrictions over the volume of wastewater to be treated.

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