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A Bilevel Programming Approach to Optimize C-phycocyanin Bio-production under Uncertainty

E. A. del Rio-Chanona, D. Zhang***

*Centre for Process Systems Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK, e-mail: : a.del-rio-chanona@imperial.ac.uk**, dz1510@imperial.ac.uk*)*

Abstract: High variability and unreliable expectations on product yields substantially hinder the industrialization of microorganism derived biochemicals as they present a risk to the profitability and safety of the underlying systems. Therefore, in this work, we propose an optimization approach to determine the lower and upper product yield expectations for the sustainable production of C-phycocyanin. Kinetic modeling is adopted in this study as it is an outstanding tool for fast prototyping, prediction and optimization of chemical and biochemical processes. On the upside, parameters in bioprocess kinetic models are used as a simplification of complex metabolic networks to enable the simulation, design and control of the process. On the downside, this conglomeration of parameters may result in significant model uncertainty. To address this shortcoming, we formulate a bilevel max-min optimization problem to obtain the worst-case scenario of our system given the uncertainty on the model parameters. By constructing parameter confidence ellipsoids, we determined the feasible region along which the parameters can minimize the system's performance, while nutrient and light controls are used to maximize the biorenewable production. The inner minimization problem is embedded by means of the optimality conditions into the upper maximization problem and hence both are solved simultaneously. Through this approach, we determined pessimistic and optimistic scenarios for the bioproduction of C-phycocyanin and hence compute reliable expectations on the yield and profit of the process.

Keywords: Uncertain dynamic systems, optimal control, bilevel programming, bioprocess optimization.

1. INTRODUCTION

C-phycocyanin is a blue antenna pigment used to enhance the photosynthetic efficiency of microorganisms such as cyanobacteria and red algae (Eriksen 2008). Because of its distinctive anti-oxidant, neuroprotective and anti-inflammatory properties, it has been recognized as a high-value bioproduct with great potential in pharmaceutical industry (Chen et al. 2013; Kuddus et al. 2013; Romay et al. 2003). In addition, it can be commercialized in cosmetic and food industries since it is a natural alternative to toxic synthetic pigments (Chen et al. 2013). Although C-phycocyanin can be synthesized by different microorganisms, currently *Arthrospira platensis*, a type of cyanobacteria, is considered as the primary species for C-phycocyanin production due to its high phycocyanin content which can accumulate up to 18% of cell dry weight (del Rio-Chanona et al. 2016).

So far, extensive research has been investigated to identify the metabolic mechanisms of C-phycocyanin synthesis in *A. platensis*. For example, several studies have been conducted recently to demonstrate that nitrate concentration and illumination intensity are the most important factors determining both the content and the productivity of phycocyanin (Xie et al. 2015; del Rio-Chanona et al. 2015). It is claimed that as phycocyanin is a light-harvesting pigment, a lower light intensity can stimulate its accumulation since phycocyanin can help cyanobacteria to collect light energy for their photosynthesis (Kuddus et al. 2013; Sun et al. 2006). Meanwhile, effects of nitrate concentration on phycocyanin production have also been well studied, and it is declared that the presence of nitrate is necessary for phycocyanin accumulation, as phycocyanin is an intracellular nitrogen storage which can be consumed by cells in nitrogen-limiting conditions (Eriksen 2008; Chen et al. 2013).

However, to accomplish the industrialization of phycocyanin production, two requirements must be satisfied. Firstly, it is necessary to identify the optimal operating conditions for phycocyanin synthesis and biomass growth so that the process efficiency can be maximised. Secondly, a final phycocyanin content higher than 10% of cell dry weight has to be guaranteed; otherwise the cost of pigment downstream separation will be significantly increased. In order to address the two requirements, advanced mathematical models have become an indispensable tool to determine the process optimal operating conditions for long-term cyanobacteria biomass cultivation and C-phycocyanin production.

Recently, a kinetic model capable of simulating cyanobacterial biomass growth and phycocyanin production under different light intensities and nitrate concentrations has been proposed (del Rio-Chanona et al. 2015). However, given the fact that a kinetic model is a simplification of a highly complex metabolic network which involves a significant amount of metabolic reactions, the uncertainty of a kinetic model is in general large and the process optimization results heavily rely on the accuracy of the

model. As a result, for microorganism related fermentation processes, it is essential to take into account the effects of model uncertainty on process prediction and optimal control. At present, significant attention has been given to process analysis, control and design with the embedding of model uncertainty (Pollock et al. 2013; Biwer et al. 2005; Rosengart et al. 2017), and different methods such as ensemble modeling has been adopted to optimize the performance of traditional fermentation processes (Guterman & Ben-Yaakov 1990; Liu & Gunawan 2017).

Nonetheless, few efforts has been made on microalgae derived biorenewables production systems, leaving an unresolved issue for the synthesis of highly demanded biochemicals such as renewable biofuels and sustainable high-value bioproducts (*e.g.* food supplement). Therefore, in order to address this challenge, in this study C-phycocyanin is selected as the representative and a bilevel programming framework is designed to optimize its production under a fed-batch long-term operation system.

2. CYANOBACTERIAL C-PHYCOCYANIN PRODUCTION SYSTEM

2.1 Process dynamic model

In our recent work, a kinetic model has been constructed to simulate both *A. platensis* biomass growth and C-phycocyanin production (del Rio-Chanona et al. 2015). This model is therefore adopted in the current study for prediction and optimization purposes. A brief introduction of this model is listed in this section.

The kinetic model is modified from the Monod model to simulate biomass growth (Eq. (21)), nitrate consumption (Eq. (22)), and C-phycocyanin production (Eq. (23)). In these equations, the specific biomass growth rate, r_M, is assumed to be a function of nitrate concentration and light intensity (Eq. (24)). Effects of light intensity on biomass growth and C-phycocyanin can be simulated by the Abia model shown in Eq. (25) and Eq. (26), respectively, since the Abia model is able to capture the performance of both photo-limitation and photo-inhibition, as well as photo-saturation.

$$\frac{dx}{dt} = r_M x - u_d x \tag{21}$$

$$\frac{dn}{dt} = -Y_{NO} r_M x \tag{22}$$

$$\frac{dq}{dt} = kx - \frac{k_d q}{n + K_{Np}} \tag{23}$$

$$r_M = u_0 \frac{n}{n + K_n} \tag{24}$$

$$u_0(I(z)) = u_m \frac{I(z)}{I(z) + k_s + I(z)^2 / k_i} \tag{25}$$

$$k(I(z)) = k_m \frac{I(z)}{I(z) + k_{sp} + I(z)^2 / k_{ip}} \tag{26}$$

where x is biomass concentration, u_0 is cell specific growth rate coefficient, N is nitrate concentration, K_n is nitrate half-velocity coefficient, K_{Np} is nitrate half-velocity coefficient for phycocyanin consumption, u_d is cell specific decay rate, Y_{NO} is nitrate yield coefficient, q is phycocyanin content in cells, k is phycocyanin production constant and k_d is phycocyanin consumption constant. u_m is maximum specific growth rate, I is light intensity, k_s is light saturation term and k_i is light inhibition term. k_m is maximum phycocyanin accumulation constant, k_{sp} is light saturation term for phycocyanin synthesis and k_{ip} is light inhibition term for phycocyanin synthesis.

Furthermore, due to the presence of light attention, local light intensity in the photobioreactor (PBR) is not uniform. To account for the severe light attenuation observed in pervious experiments, the modified Lambert-Beer's law in Eq. (26) is adopted to estimate local light intensity which is attenuated by bubble scattering and cell absorption.

$$I(z) = I_0[\exp(-(\tau x + K_a)z) + \exp(-(\tau x + K_a)(L - z))] \tag{27}$$

where I_0 is incident light intensity, τ is cell absorption coefficient, K_a is bubble reflection coefficient, z is the distance from light source, and L is the width of the PBR.

In our recent work (del Rio-Chanona et al. 2015), an average growth rate for u_0 and k was calculated. For computational tractability, in this work we calculate the average light intensity (I_{av}) received by cells by integrating Eq. (27) local light intensities over the volume of the reactor, which is then used to substitute local light intensities ($I(z)$) presented in Eq. (25) and Eq. (16).

Moreover, given the fact that nitrate inflow rate (F_N) is a control in this study, while the model was originally design to simulate a batch process, a new term, $F_N \cdot N_{in}$ is added on the right-hand-side of Eq. (22) so that the model can be used to simulate the fixed-volume fed-batch process. The influent nitrate concentration (N_{in}) is assumed to be 500 mM and the fed-batch is designed to be a 16-day process.

2.2 Process description

To maximize the production of C-phycocyanin a 16-day fed-batch process was selected as the production mode for operation, and dynamic equations were modified accordingly. This would allow to treat the nitrate inlet flow and the light intensity as control variables, and hence we can formulate an optimal control problem. To maintain this scenario as close to reality as possible, controls where only allowed to vary once per day during a 16 day computational experiment.

3. PARAMETER ESTIMATION AND CONFIDENCE ELLIPSOIDS

3.1 Parameter estimation

In this section we outline the procedure followed to estimate the parameters in (31) for the model in equations (21)-(26).

$$p = (u_m, K_N, u_d, Y_{NO_3}, k_d, K_{N_p}, k_m, k_s, k_i, k_{sp}, k_{ip}, \tau, K_a) \quad (31)$$

To estimate the parameters in (31), the following nonlinear least-squares optimization problem is formulated.

$$\begin{aligned} &\min_p \sum_{i=1}^{N} (\hat{y}_i - y(t_i, p))^T \Lambda_i (\hat{y}_i - y(t_i, p)) \\ &s.t. \\ &\frac{dx}{dt} = f(x(t), p) \\ &p_{lb} \le p \le p_{lb} \\ &x(0) = x_0 \\ &t_0 \le t \le t_f \end{aligned} \quad (32)$$

The output variables once the ODE system is solved are labelled y, while the experimental data is labelled $\hat{y}$, p_{lb} and p_{ub} are the upper and lower bounds of parameters, and Λ is the inverse of the covariance matrix.

Given that the parameter estimation problem is nonconvex, and to avoid shallow local minima, a multi-start framework was implemented. One hundred initial starting points were generated by a Sobol sequence and the estimated parameters that produced the lowest objective function was chosen.

3.2 Confidence Ellipsoids

Joint confidence regions were determined by considering all simultaneous linear combinations of the parameters, computed by:

$$(p - p^*)^T \Lambda (p - p^*) \le \chi^2_{1-\alpha, NP} \quad (33)$$

where p^* is the optimal parameter solution determined by (23), and the right-hand side is the standard value for the Chi-square test given the number of parameters and a 95% level of confidence.

4. OPTIMIZATION OF PROCESS PRODUCTION

An optimal control problem was formulated to optimize the production of C-phycocyanin in a fed-batch operation mode. To maximize the process production two control variables where used, light intensity and nitrogen inflow rate. The resulting dynamic optimization problem is the following:

$$\begin{aligned} &\max_{Fin(t), L(t)} q(t_f) \\ &s.t. \\ &\frac{dx}{dt} = f(x(t)) \\ &x(0) = x_0 \\ &Fin^{\min} \le Fin(t) \le Fin^{\max} \\ &L^{\min} \le L(t) \le L^{\max} \\ &t_0 \le t \le t_f \end{aligned} \quad (41)$$

The solution of (41) would result in the optimal control sequences for *Fin*(*t*) and *L*(*t*).

Problem (41) is discretized through orthogonal collocation over finite elements, where the collocation points are placed according to a fifth order Radau quadrature. This yields the resulting nonlinear programming (NLP) problem:

$$\begin{aligned} &\max\ q(N_E) \\ &s.t. \\ &\dot{x}_{i,j} = f_c(x_{i,j}, \dot{x}_{i,j}) \\ &x_{i,j} = x_{i-1,K} + h_i \sum_{l=1}^{K} \Phi_l(\tau_j) \dot{x}_{i,l} \\ &x_{i,0} = x_{i-1,K} \\ &x_{1,0}(t_0) = x_0 \\ &Fin^{\min} \le Fin_{i,j} \le Fin^{\max} \\ &L^{\min} \le L_{i,j} \le L^{\max} \\ &t_0 \le t \le t_f \end{aligned} \quad (42)$$

where q is C-phycocyanin production, N_E is the number of finite elements, and we take x to represent the states in the differential equation model.

5. WORST-CASE SCENARIO OPTIMIZATION

5.1 Bilevel programming approach

A general approach to determine worst-case scenarios in complex optimization problems is to sample different scenarios and then either derive statistical moments on the solution, or use the worst scenario sampled to determine the possible worst-case scenario. In this work, we present a strategy that avoids statistical inference or scenario sampling. The bilevel max-min approach enables us to formulate a problem that would yield the worst-case scenario of the dynamic optimization in the system. In this case, given that we have no strict bound or inequality constraints that our system is likely to violate, the worst-case is the scenario with the lowest product production even when the system is optimized. For this, we maximize the production of C-phycocyanin by using light and nitrate inlet as control parameters. Furthermore, we assume the system will select the worst possible set of parameters that are within the 95% confidence intervals computed by (33). This bilevel optimization problem is the following:

$$
\begin{aligned}
&\max_{Fin(t),L(t)} q(t_f)\\
&s.t.\\
&\frac{dx}{dt} = f(x(t))\\
&x(0) = x_0\\
&Fin^{\min} \le Fin(t) \le Fin^{\max}\\
&L^{\min} \le L(t) \le L^{\max}\\
&t_0 \le t \le t_f\\
&\quad \min_p q(t_f)\\
&\quad s.t.\\
&\quad (p - p^*)^T \Lambda (p - p^*) \le \chi^2_{1-\alpha,NP}
\end{aligned}
\tag{51}
$$

Problem (51) can be reformulated by embedding the necessary and sufficient conditions for optimality of the inner minimization problem into the outer optimization problem. This formulation of a bilevel problem is referred as mathematical programs with complementarity constraints (MPCC). MPCC have a less complicated structure than the original Bilevel problems, particularly, the feasible sets are always closed (Allende & Still 2013). This reformulation yields the following single-level problem:

$$
\begin{aligned}
&\max_{Fin(t),L(t)} q(t_f)\\
&s.t.\\
&\frac{dx}{dt} = f(x(t))\\
&x(0) = x_0\\
&Fin^{\min} \le Fin(t) \le Fin^{\max}\\
&L^{\min} \le L(t) \le L^{\max}\\
&t_0 \le t \le t_f\\
&\nabla_p L(p^*) = \nabla_p F(p^*) - \mu \nabla_p G(p^*) = 0\\
&\mu G(p^*) = 0\\
&\mu \ge 0\\
&\nabla^2_{pp} L(p^*) \succeq 0
\end{aligned}
\tag{52}
$$

where μ is the Lagrange multiplier for the constraint on the parameters feasible region, delimited by the confidence ellipsoid and G and F are

$$G = (p - p^*)^T \Lambda (p - p^*) - \chi^2_{1-\alpha,NP} \tag{53}$$

$$F = q(t_f) \tag{54}$$

When embedding the lower bilevel problem into the upper problem by the conditions of optimality it is advisable to use the Fritz-John (FJ) necessary conditions (Allende & Still 2013). However, given that we do not have equality constraints for the lower problem, the Karush-Kuhn-Tucker (KKT) and the FJ necessary conditions are the same.

Furthermore, we consider the smoothing approach (Allende & Still 2013) where we replace the complementary equation:

$$\mu G(p^*) = 0 \tag{55}$$

by

$$\mu G(p^*) = \varepsilon \tag{56}$$

where $\varepsilon > 0$ is a small perturbation parameter.

Notice that for us to obtain expression (54) we would have to integrate (23) which itself is a function of the integral of (26). This becomes problematic, and hence we replace (54) with

$$F = \int_{t_0}^{t_f} \frac{dq}{dt} dt \tag{57}$$

Given our orthogonal collocation formulation, (57) is implemented in NLP form as:

$$F = \sum_{=1}^{N_E} f_q(x_{i,j}, \dot{x}_{i,j}) \tag{58}$$

where

$$\dot{q}_{i,0} = \dot{q}_{i-1,0} = f_q(x_{i,j}, \dot{x}_{i,j}) \tag{59}$$

where in (59) q represents our state of total phycocyanin production and x correspond to all states involved in (23).

Finally, let us note that (23) is only twice differentiable for parameters k_{sp}, k_{ip}, K_a, τ, *and* K_{Np}, hence they are the only parameters considered as optimization variables.

6. IMPLEMENTATION

Optimization problems in this work were implemented in a Python programming environment, using Pyomo as an interface for the optimization solver IPOPT (Wächter & Biegler 2006). The value of ε vas set to be 10^{-6}. Furthermore, solving (52) directly was found difficult due to inadequate starting points for the optimization problem. Therefore (41) was first solved to obtain preliminary initialization points, later, to ameliorate convergence issues with the numerical solution of (52) expressions

$$\nabla_p L(p^*) = \nabla_p F(p^*) + \mu \nabla_p G(p^*) = 0 \tag{61}$$

$$\mu G(p^*) = \varepsilon \tag{62}$$

which contain bilinear and trilinear terms substituted by their linearized form (Adjiman et al. 1998). Subsequently, linearized expressions of (61) were introduced one-at-a-time for each parameter in question to update the initial guesses for the optimizer. Finally, (61) and (62) where re-introduced in the overall problem, and an optimal solution to (52) was attained. In addition to this approach, linearizing the bilinear terms in (61) and (62) by a mixed-integer approach was also

implemented, however the problem was found more difficult to converge than with the convex relaxation approach.

7. RESULTS AND DISSCUSSION

7.1 Best-case and worst-case scenarios

To obtain an optimistic scenario of the process optimization, (41) is solved, however, parameters in (31) were also assigned as optimization variables, hence helping the maximization of the bioproduct.

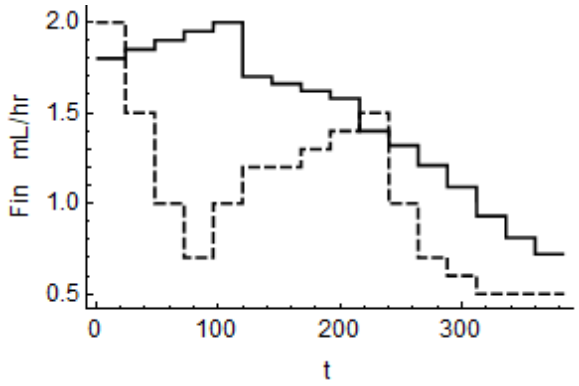

Fig. 1. Optimal nitrate inlet input for the optimistic and pessimistic scenarios. Solid line: optimistic scenario control. Dotted line pessimistic scenario control.

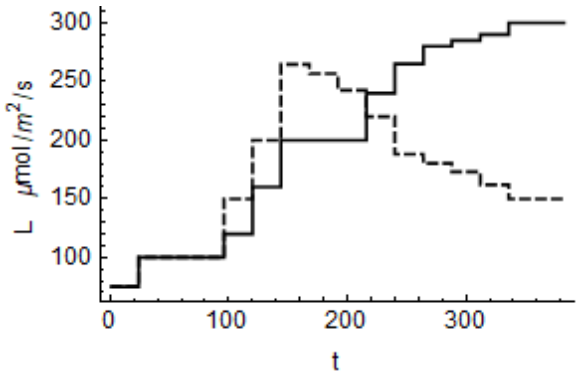

Fig. 2. Optimal light input for the optimistic and pessimistic scenarios. Solid line: optimistic scenario control. Dotted line pessimistic scenario control.

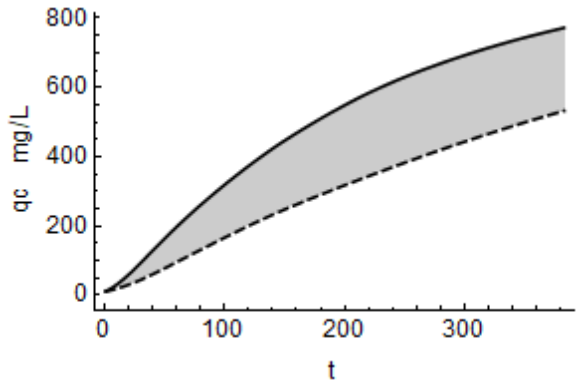

Fig. 3. Optimal production for the optimistic and pessimistic scenarios. Solid line: optimistic production. Dotted line pessimistic scenario production.

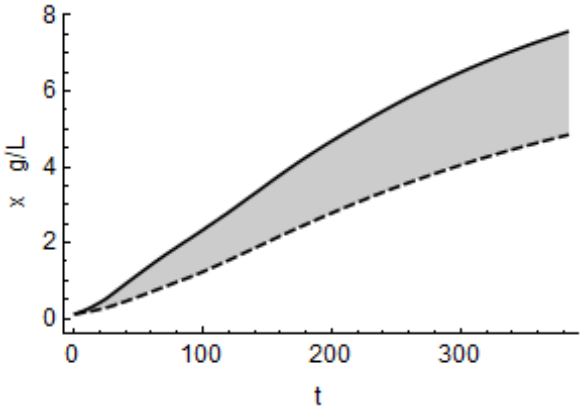

Fig. 4. Biomass dynamic behaviour for the optimistic and pessimistic scenarios. Solid line: optimistic scenario control. Dotted line pessimistic scenario control.

7.2 Discussion

The parameter values for both scenarios are listed in Table 1.

Parameter	Pessimistic scenario	Optimistic scenario
k_{sp}	37.328	11.323
k_{ip}	197.78	800.0
K_a	3.799	0.0
τ	73.381	48.382
K_{NP}	18.372	16.892

From Fig. 3 and Fig. 4, it can be seen that there is a significant change in the potential production on the process, which is summarized in Table 2. This highlights the fact that an incorrect estimation of the best and worst production yields can significantly endanger the safety and economics of the process.

	Biomass conc.	C-phycocyanin production
Optimistic scenario	4.8	773.2
Pessimistic scenario	7.5	533.9

Furthermore, it is also worth noticing that there is a significant difference between the optimal control inputs for light and nutrient supply, depending on the dynamic model parameters chosen. This means, that as expected, different parameter sets will result in different optimal control inputs to maximize the bioproduct. Furthermore, given the wide confidence intervals, more experimental measurements are needed to narrow the varying range of the controls. This highlights the need for an online identification and optimization strategy to be put in place (*e.g.* MPC, EMPC) so that process optimization can be performed effectively.

In Table 1 we can see that k_{sp}, which is the light saturation term, is higher in the pessimistic scenario than in the optimistic scenario. A system with a higher light saturation term is not able to use light to produce phycocyanin as efficiently as one that has a lower light saturation term. Moreover, the light inhibition term (k_{ip}) is higher in the optimistic scenario, which means that when there is a high light intensity, it will not inhibit the production of phycocyanin as much as that in a system with a lower value of k_{ip}, such as the pessimistic scenario.

K_a and τ regulate how much the culture scatters and absorbs light, respectively. In both cases the pessimistic scenario has a higher value, which diminishes the amount of light that can be utilized for phycocyanin production. Furthermore, K_{Np} can be seen to have practically the same value in both cases, which reflects the low sensitivity of K_{Np} to phycocyanin production, and that other parameters can inhibit the systems performance more severely. Thus, it is important to identify the correct value of other parameters through model-based experimental design method in future study.

There are still shortcomings of the present methodology that will be addressed in future work. In first instance, the computational tractability of the current problem should be improved. In addition to this, there is no guarantee that the follower optimization problem converges to a global minimum, which may lead to an overestimation of the worst-case scenario.

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