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Dynamic Flux Balance Analysis in Cyanobacteria for Ethanol Production with Simultaneous Optimization Approaches

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Abstract

In this work we address dynamic optimization of ethanol production by Dynamic Flux Balance Analysis in an engineered cyanobacterium with autotrophic growth (i.e., using CO_2 as substrate). The photobioreactor optimization model is integrated to the metabolic network one by replacing the inner problem by its first order optimality conditions. Complementarity constraints that arise associated to the optimality conditions are efficiently handled with a penalty function formulation. Numerical results suggest the possibility to activate the ethanol production pathway after 20 h in the batch run to enhance ethanol production.

Keywords: dynamic flux balance, bioethanol, cyanobacteria, dynamic optimization

1. Introduction

Considerable effort is being devoted to obtain third generation biofuels from algae. Algal biofuels constitute sustainable alternatives, as they uptake carbon dioxide as the carbon source, have higher yields than other terrestrial biomass feedstock, and can be grown with non-fresh water sources without requiring high-value arable land. Their photoautotrophic growth also enables capture of industrial carbon dioxide emissions to reduce greenhouse gasses pollution. In particular, cyanobacteria are an abundant and diverse group of ancient autotrophic prokaryotes that perform oxygenic photosynthesis. Optimizing photosynthetic organisms for biotechnological purposes will therefore require a systems understanding of photosynthetic processes (Nogales et al., 2012).
During the last decade, a few authors have addressed the study and design of metabolic networks by dynamic flux balance analysis (DFBA). In this approach, a bilievel optimization problem is formulated with the dynamic bioreactor model at the outer optimization level and the metabolic network linear model as the inner level. Raghunathan et al. (2003) addressed data reconciliation and parameter estimation in metabolic flux balance models by formulating this type of bilevel optimization problem and reformulating it as a single level one by replacing the inner LP by its optimality conditions; they solved the resulting NLP with an interior point method. Gadkar et al. (2005) proposed DFBA for glycerol and ethanol production from E. coli, solving the bilevel optimization problem in Matlab. Laiglecia et al. (2013) have carried out parameter estimation for ethanol producing cyanobacteria by reformulating the bilevel problem as a single level one.

In this work we address dynamic flux balance analysis, to optimize ethanol production from the same genetically modified cyanobacteria. The control variables of the dynamic optimization problem are batch temperature, light intensity and phosphate concentration in the culture medium. The model includes two major components: (a) a dynamic model with mass balances for biomass, ethanol, nitrate, phosphate, internal nitrogen and phosphorus (Laiglecia et al., 2013), and (b) a steady state metabolic Linear Programming (LP) model of the central carbon metabolism. The biomass equation includes limiting functions for light, temperature and nutrients, kinetics of growth inhibition by ethanol toxicity and the decrease in the available light by biomass concentration increase. Numerical results suggest activating the ethanol production pathway 20 h after the beginning of the batch run to increase ethanol production in around 26 %.

2. System Description

We address the enhancement of ethanol production by a mutant strain of the cyanobacterium Synechocystis sp. PCC 6803 obtained by Vidal (2009). This modified strain harbors the heterologous genes pdc and adhII from the ethanolgenic bacterium Zymomonas mobilis, to create an ethanol production pathway. The pdc and adhII genes encode, respectively, the enzymes pyruvate decarboxylase (pdc), which catalyzes the non-oxidative decarboxylation of piruvate (PYR) to produce acetaldehyde (ACAL) and CO2, and alcohol dehydrogenase II (adhII), which participates in the reduction of acetaldehyde to ethanol. The new pathways are as follows:

pdc:	pyruvate decarboxylase	$PYR \rightarrow ACAL + CO_2$
adh:	alcohol dehydrogenase isoenzyme IV	$ACAL + NADH \leftrightarrow ETH + NAD$

In previous work, Deng and Coleman (1999) inserted these heterologous genes in *Synechococcus* sp. strain PCC 7942; Dexter and Fu (2009) inserted these genes in *Synechocystis* PCC 6803 under the control of the constitutive promoters from the *rbcLS* and *psbAII* genes. In this paper, we work with the cyanobacterium *Synechocystis* sp. PCC 6803 strain obtained by Vidal (2009), in which these genes have been introduced under the control of the gene *petE* promoter. Expressing the operon *pdc-adhII* under the control of this promoter, which is induced by copper, allows the induction of the ethanol system production when this is required or, otherwise, kept inactive.

3. Dynamic Flux Balance Analysis

Much research has been devoted to modeling of metabolic networks during the last two decades. The most popular approaches are based on the pseudo steady state assumption for the metabolic network, which is supported by the fact that intracellular, enzyme catalysed reactions have a relaxation time of milliseconds, which is very fast as compared to the relaxation time of cellular growth, normally on the order of hours. Within these approaches, Flux Balance Analysis (FBA) is a genome-scale constraint-based modeling approach for metabolic networks, where steady state mass balances corresponding to metabolic fluxes (reactions) around each node (metabolite) are formulated, rendering an underdetermined systems of linear equations. In FBA, a linear programming problem (LP) is formulated where the objective function is the maximization of the growth rate. Solving the LP problem gives the metabolic flux distribution of the cell. However, this approach allows a static description of the metabolic network, which is appropriate for continuous cultures, but does not account

for regulation and does not provide description of dynamic properties. In this sense, Dynamic Flux Balance Analysis allows modeling the interaction between the cellular metabolism and the environment, by keeping a linear model that describes intracellular reactions and dynamic equations for extracellular reactions. The LP problem for the metabolic network is embedded within an outer optimization model that takes into account dynamic mass balances for main substrates, products and biomass at the bioreactor level, allowing the inclusion of kinetic expressions and resulting into a bilevel optimization problem. It is a case of spatial and temporal multiscale problem, which we reformulate as a single level optimization problem by replacing the inner optimization problem by its first order optimality conditions (Karush Kuhn Tucker, KKT, conditions), as detailed in Section 6.

4. Bilevel optimization problem

In the outer optimization problem, we formulate dynamic mass balances for biomass, nutrients and ethanol at the batch photobioreactor level. We also include mass balances for internal phosphorus and nitrogen to model the storage of the main nutrients, which are described in Laiglecia et al. (2013), as well as the model parameters. The net growth rate (μ) is calculated affecting the maximum growth rate (v^*_{growth}), which is the objective function for the inner optimization problem, by limiting functions for ethanol concentration (E) and temperature (T) as follows:

$$\mu = v^*_{growth} f(T) f(E) \tag{1}$$

$$f(E) = \frac{1}{1 + \frac{E}{KI}} \tag{2}$$

$$f(T) = \frac{T}{T_{opt}} exp\left(1 - \frac{T}{T_{opt}}\right) \tag{3}$$

The objective is to maximize ethanol concentration along the time horizon, re-written as minimization of the square difference between ethanol concentration (*E*) and a desired value (*Esp*), for a better performance of the optimization algorithm, as follows:

$$min \int_0^{tf} (E - Esp)^2 dt \tag{4}$$

The internal metabolism is represented by an LP problem that maximizes biomass growth rate (v^*_{growth}) subject to a linear homogeneous system for the internal metabolic fluxes. The metabolic network model includes 50 metabolites and 57 metabolic reactions which represent the central carbon metabolism of *Synechocystis* that comprises the Calvin cycle (CO_2 fixation), Krebs and pentose phosphate cycles, glycolysis, pyruvate metabolism and transport system. The model also includes the reactions presented in Section 2, corresponding to the heterologous genes *pdc* and *adh*. The intracellular and extracellular models are linked by biomass growth rate (v^*_{growth}), absorbed photon flux (v_{APF}) and phosphorus uptake rate by the microorganism (v_{PO_4}). The model includes a limiting function for the light uptake, *f*(*I*), that takes into account

the decrease of light availability (*I*) by biomass accumulation in the reactor. The phosphate uptake incorporates a kinetic expression *f(N)* depending on both external (*Pmax, Pmin*) and internal phosphorus concentration (*PI*). Bounds over these fluxes (Eqs. 5-6) represent additional constraints for the outer problem, being v_{APF}^{*} and v_{PO4}^{*} , fixed upper bounds.

$$v_{APF} \leq v_{APF}^{*} f(I) \tag{5}$$

$$v_{PO_4} \leq v_{PO_4}^{*} f(N) \tag{6}$$

$$f(N) = \frac{PI - P_{min}}{P_{max} - P_{min}} \tag{2}$$

$$f(I) = \frac{I_o}{I_{opt}} exp\left(1 - \frac{I_o}{I_{opt}}\right) \tag{3}$$

$$I_o = \frac{1 - exp(K_{ext}\, p\, X)}{(K_{ext}\, p\, X)} \tag{4}$$

5. Solution strategy

The reformulation of the previously described bilevel optimization problem into a single level one is carried out by replacing the inner optimization problem (intracellular model) by its first order optimality conditions (KKT), as follows:

$$\nabla v_{growth}(v) + A^T \lambda + \mu^U - \mu^L = 0 \tag{10}$$

$$Av = 0 \tag{11}$$

$$v - v^U + s^U = 0 \tag{12}$$

$$-v + v^L + s^L = 0 \tag{13}$$

$$\mu^U \perp s^U \tag{14}$$

$$\mu^L \perp s^L \tag{15}$$

$$s^U, s^L, \mu^U, \mu^L \geq 0 \tag{16}$$

where metabolic flux lower and upper bounds are converted into equalities by adding positive slack variables s^U, s^L, respectively. Kuhn Tucker multipliers for the nonnegativity condition on slack variables are μ^U, μ^L, respectively. The single level dynamic optimization problem is solved by a simultaneous optimization approach, in which state and control variables are approximated by piecewise polynomials over finite elements. Differential and algebraic equations are discretized over these finite elements, rendering a large scale nonlinear programming problem (NLP).

6. Discussion of results

In previous work, we have carried out fermentations with the genetic engineered strain Synechocystis sp. PCC 6803, which was cultivated in BG-11 medium at 30 °C under continuous light (100 μE m-2 s-1) and air bubbling enriched with 1 % CO2, which is considered as a CO2 rich medium. Liquid batch cultures were performed by duplicate for wild type and ethanol mutant strains for 73 hours from the beginning of the exponential growth phase of growth. Biomass was estimated by OD, Chlorophyll a concentration and total organic carbon. Experimental data from the fermentations have been used to estimate main parameters for the extracellular model (Laiglecia et al., 2013). In this work, we carry out dynamic optimizations to maximize ethanol production, while maximizing cell growth in the inner intracellular model. The discretized NLP is formulated in GAMS (Brooke et al., 2012) using an automatic reformulation of complementarity constraints (14), (15) with the penalty formulation provided by the NLPEC meta-solver, and solving the resulting NLP with CONOPT3. Control variables are bioreactor temperature (T), light intensity (I) and phosphate feed flowrate (which is considered negligible as compared to tank volume). We have considered the addition of phosphate to increase ethanol production because the pdc catalyzed reaction (for acetaldehyde production from pyruvate) is decreased to negligible values after 40 hours (see Fig. 1) in simulations related to experimental conditions. The problem has 58,351 variables and 88,483 constraints when discretizing with 73 finite elements and two collocation points. The CPU time is 297 s. Figures 1 to 4 show DFBA results and their comparison with simulations for the described fermentation experiments.

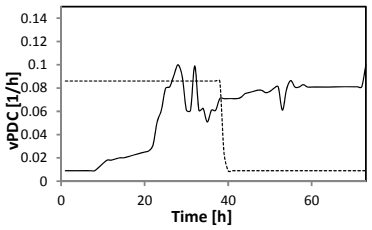

Figure 1. Reaction rates for the intracellular pdc catalyzed reaction. (⋯) Parameter estimation simulation (—) Optimal profile

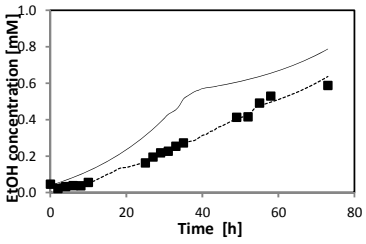

Figure 2. Extracellular ethanol concentration profiles. (■) Experimental data (⋯) Parameter Estimation simulation (—) Optimal profile

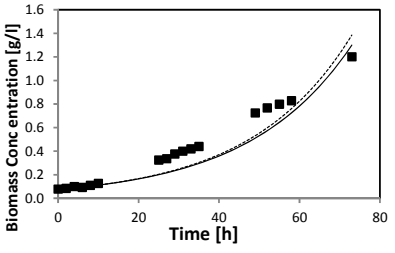

Figure 3. Biomass concentration. (■) Experimental data (⋯) Parameter estimation simulation (—) Optimal profile

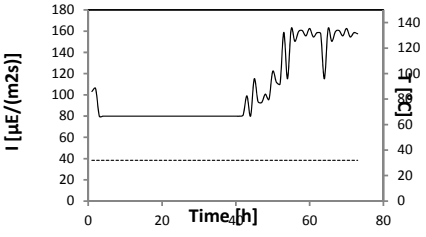

Figure 4. Control Variables for the Optimization problem. (⋯) Temperature (—) Irradiation

Experimental work has been carried out using the genetic modified strain, and activating the Pet promoter from the very beginning of the runs (with copper); i.e., enabling ethanol production path throughout the entire time horizon. However, numerical results obtained with DFBA suggest modifications in the metabolic network during the fermentation, with the consequent increase in ethanol production. Figure 1 shows the pdc catalyzed reaction (for acetaldehyde production and hence ethanol production, from pyruvate) in both the experimental conditions and after optimization. It can be seen that this pathway in the optimized network should be activated after 20 hours of fermentation, with the consequent increase in ethanol production. Extracellular metabolite concentrations for ethanol are shown in Fig. 2. It can be seen that during the analyzed time horizon ethanol concentration is increased 26 % with respect to the simulated values. Figure 3 shows experimental, simulated and optimal values for biomass concentration profiles; it can be noted that ethanol production increase does not affect biomass growth, as it has been previously shown in experiments carried out by Vidal (2009). Figure 4 shows optimal profiles for batch temperature, which is kept constant at 32 C and light intensity, which is constant at 80 μE/(m2.s) up to 40 h and increases to double its value by the end of the fermentation. In this last case, the irregular shape could be due to the fully discretization approach.

7. Conclusions

In this work we have integrated a photobioreactor model with a metabolic network for an engineered cyanobacterium for ethanol production by reformulating the bilevel problem replacing the inner optimization problem by its KKT conditions. Numerical results suggest possible modifications to the metabolic network during the runs to enhance ethanol production, using CO_2 as substrate for fermentations.

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