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Batch Control of Genetic Alterations for Optimal Metabolic Engineering

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Abstract— **Metabolic engineering manipulations can be performed in an optimal manner to maximize desired cellular properties. In prior work [1], a bilevel optimization framework was developed to demonstrate that temporal genetic manipulations yield optimal productivity. In this work, the bilevel optimization framework is coupled with control algorithms to determine the genetic manipulation strategies in practical bioprocess situations. Ethanol production in an anaerobic batch fermentation of $Escherichia\ coli$ in two case studies are considered. In the first, the bilevel optimization framework is augmented to incorporate a penalty for longer time of operation. The framework successfully optimizes the batch time along with the genetic manipulations for maximizing the desired objectives. In the second, the bilevel optimization framework is coupled to a parameter estimation algorithm to compensate for plant-model mismatch. Starting from extreme initial guesses for the unknown growth inhibition constant, the framework converges to the optimal solution within 4 batches.**

I. INTRODUCTION

Traditional biochemical engineering has focused on the design and operation of bioreactors. However, in the past decade, metabolic engineering and metabolic modeling have integrated into biochemical engineering [2]. Metabolic engineering involves the application of recombinant DNA methods to manipulate metabolic networks for the directed improvement in metabolite and protein production capabilities [3]. Along with macroscopic considerations such as process design and control, molecular manipulations such as genetic perturbations can be achieved in a systematic fashion [4], [5]. Recent advances in recombinant DNA and other molecular biology techniques enable well-defined genetic manipulations such as deletion, amplification, down-regulation and modification of the genes that encode the critical enzymes involved in the process [6], [7]. Advances in such techniques have lead to the commercialization of biotechnology based products such as pharmaceuticals, health-care and agricultural products. The optimal control, both at the genetic and bioreactor level, of these processes is of considerable interest and a challenging research area.

Gadkar et al. [1] have demonstrated that optimal metabolic engineering requires a temporal control of gene expression. A bilevel optimization framework was developed that determines the optimal flux profile corresponding to the key reactions for maximization of the desired product concentration. The key reactions are identified $a\ priori$ using the 'OptKnock' approach [8]. The utility of the framework was demonstrated for glycerol and ethanol production in $Escherichia\ coli$. In a batch fermentation, glycerol production increased by 30% with temporal genetic manipulation compared to the case when the genetic change was introduced at the start of the batch. In the case of ethanol, an optimal manipulated flux through the acetate secretion pathway increased production by 42% and 91% compared to the cases with fully inactive and fully active fluxes, respectively. It was also demonstrated that the optimal solution of the temporal genetic alterations was dependent on operating conditions and system dynamics. In case of glycerol production the optimal solutions varied considerably with oxygen availability. Likewise, variations in the optimal acetate flux profiles were observed by introducing growth inhibition due to high ethanol concentrations.

In this work, the bilevel optimization framework is used to determine the optimal manipulation profiles for batch fermentation for ethanol production. The focus is primarily on two case studies. In the first, a penalty is introduced for higher operation time. The optimal batch time is determined along with the optimal genetic alteration profile by the bilevel optimization. In the second case study, plant-model mismatch is introduced by unmodeled system dynamics. The bilevel optimization framework is coupled with a parameter estimation algorithm. With each successive iteration, the parameter estimate is corrected forcing the bilevel optimization towards the optimal solution.

II. SYSTEM

The system of study in this work is the anaerobic batch fermentation of $Escherichia\ coli$. Under anaerobic growth with glucose as the substrate, $Escherichia\ coli$ produces a number of reduced by-products including acetate, ethanol, lactate and succinate. The reactions involved in the production of the by-products are shown in Fig. 1. The acetate pathway is activated mainly to regenerate the ATP levels in the cell [9]. The ethanol and lactate branches lead to oxidation of NADH [10]. The lactate branch competes with the ethanol branch for NADH oxidation inside the cell. An ldh knockout strain (gene coding for lactate dehydrogenase) inactivates the lactate branch forcing increased NADH oxidation via the ethanol branch. This results in an increased ethanol flux [11]. Furthermore, the ldh knockout does not have a significant effect on growth rate. Inactivation of the acetate branch ($ackA$ knockout) further increases the ethanol flux. The reason for this is that the entire flux

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TABLE I

STEADY STATE GROWTH RATE AND ETHANOL FLUX UNDER ANAEROBIC FERMENTATION OF $E.\ coli$ FOR A GLUCOSE UPTAKE FLUX OF 10 MMOL G-BIOMASS^{-1} H^{-1}

Strain	Growth rate (h^{-1})	Ethanol flux (mmol g-biomass^{-1} h^{-1})
ldh knockout	0.3112	06.2
ldh+$ackA$ knockout	0.2370	12.5

entering the acetyl CoA in diverted towards the ethanol branch (Fig. 1). However in doing so ATP regeneration in the cell is affected which results in reduced growth rates. Table I shows the steady state ethanol flux and growth rates for the ldh and $ackA$ knockouts calculated $in\ silico$. Underwood et al. [12] have demonstrated that changes in the carbon partitioning between fermentation reactions and biosynthesis is critical in reduction of the time of fermentation during ethanol production. As a result, for maximizing the end ethanol production, an optimal temporal acetate flux must be determined over the batch time so that there is optimal distribution of fluxes favoring growth and ethanol production over the period of the batch.

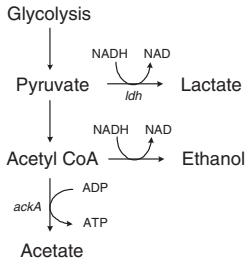

Fig. 1. Reactions involved in the production of by-products during anaerobic growth of $E.\ coli$

III. METHODS

An $in\ silico$ representation of $Escherichia\ coli$ [13], [14] including all the reactions in the central metabolism of the cell, consisting of a total of 57 internal reactions, 13 exchange fluxes and 54 internal metabolites is used in this work. The Flux Balance Analysis (FBA) approach is used to determine the metabolic flux distribution in the network [15]. The FBA solution is based on the assumption that the metabolic fluxes are distributed optimally such that a built-in cellular objective is achieved. It also assumes a steady state of all intracellular reactions, resulting in no accumulation or depletion of internal metabolites. For $Escherichia\ coli$, it has been demonstrated experimentally that this objective is maximization of the cell growth rate [16], therefore the growth rate is used as the objective in this work. Glucose is considered as the only carbon source. The maximum glucose uptake rate is pre-specified at 10 mmol g-biomass^{-1} h^{-1} and uptake is enabled via both the phosphotransferase system and the glucokinase enzyme. Unconstrained uptake of inorganic phosphate is considered and secretion fluxes for acetate, ethanol, formate, lactate, succinate, pyruvate and carbon dioxide are enabled. All batch simulations start with initial glucose and biomass concentrations of 10 mmol L^{-1} and 0.01 g L^{-1} respectively.

A. Case study I: Batch fermentation with batch time penalty

A bilevel optimization scheme is formulated to maximize the ethanol concentration at the end of the batch with a penalty for longer batch times. The optimization determines the optimal time of regulation (t_{reg}) of the acetate flux from an initial high value to a final low value and the optimal batch time. There exists a trade-off between growth and product flux that is optimized by the time of regulation. In addition, the trade-off between increasing ethanol production with higher batch times and the resulting penalty imposed is optimized by the batch time. The bilevel optimization is formulated as shown below:

$$\max_{t_{reg},t_f} P(t_f) - W(t_f - t_{base}) \tag{1}$$

$$\text{s.t. :} \left(\begin{array}{lll} \max\limits_{v_j(t)} & v^{growth}(t) & \forall \quad j \in Q \\ \text{s.t. :} & \sum\limits_{j=1}^{M} S_{kj} v_j(t) = 0 & \forall \quad k \in N \\ & \alpha_j \leq v_j(t) \leq \beta_j & \forall \quad j \in M \\ & v_j(t) = v_c^- & \forall \quad j \in U,\ t < t_{reg} \\ & v_j(t) = v_c^+ & \forall \quad j \in U,\ t \geq t_{reg} \\ & v_{pts}(t) + v_{glk}(t) = v_{glucose}^{uptake}(t) & \end{array} \right)$$

$$\begin{aligned} t_f &\leq t_f^{max} \\ \mathrm{d}P/\mathrm{d}t &= v^{product}(t)X \\ \mathrm{d}X/\mathrm{d}t &= v^{growth}(t)X \\ \mathrm{d}G/\mathrm{d}t &= -v_{glucose}^{uptake}(t)X \end{aligned}$$

The batch biological behavior is predicted using the static optimization approach (SOA) [17]. In the outer optimization, the optimal time of regulating the manipulated flux (t_{reg}) and the optimal batch time (t_f) are determined such that the objective function shown in (1) is maximized. P represents the product concentration and t_{base} represents the batch time above which the penalty in imposed. The severity

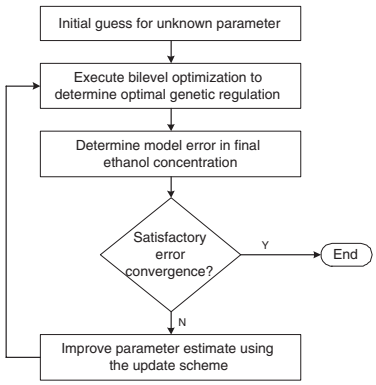

Fig. 2. Algorithm for batch control of optimal genetic regulation for unmodeled system dynamics

of the penalty depends on the weight of the penalty represented by W. The outer optimization is subject to the inner optimization at each instant over the period of the batch. This inner optimization forces the unregulated fluxes to be distributed such that growth rate is maximized. The terms α_j and β_j denote the lower and upper bounds for the fluxes. These constraints are imposed subject to the availability of information. For example, for an irreversible reaction the lower bound is taken to be zero. The manipulated flux profile determined in the outer optimization is forced in the inner optimization. The manipulated flux is maintained at v_c^- for times less than t_{reg} and v_c^+ for times greater than t_{reg}.The quantities v_c^- and v_c^+ are fixed quantities and they represent the values of the manipulated flux before and after the repression respectively. These values are predetermined and are used as fixed parameters in the bilevel optimization. The initial flux value (v_c^-) is taken to be 5 mmol g-biomass^{-1} h^{-1} (active acetate flux) and the final flux value is taken to be 0 mmol g-biomass^{-1} h^{-1} (inactive acetate flux). The maximum batch time is bound by a maximum value represented as t_f^{max}.

The bilevel optimization is evaluated for two scenarios, with and without growth inhibition due to high ethanol concentrations. The growth inhibition is modeled by reducing the maximum glucose uptake rate at higher ethanol concentrations:

$$v_{glucose}^{uptake} = v_{glucose}^{uptake\,*} \frac{1}{1 + \frac{[E]}{K_i}} \tag{2}$$

In this case study the dynamics of the growth inhibition are assumed to be known and the value of the inhibition constant K_i is taken to be 25 mmol L^{-1}.

B. Case study II: Batch control under unmodeled system dynamics

In this case study, the optimal genetic alterations are determined for batch fermentations in the presence of unmodeled system dynamics. The optimal time of regulation of the acetate flux is determined for maximizing the ethanol production in the presence of growth inhibition by high ethanol concentrations. However, in this case study the inhibition constant (K_i) is unknown. With each successive batch run, the estimate of the unknown parameter is updated. The algorithm for the batch to batch control is depicted in Fig. 2. In this case study, the batch time is fixed and only the optimal regulation time is determined by the bilevel optimization. The bilevel optimization is formulated as shown below:

$$\max_{t_{reg}} : P(t_f) \tag{3}$$

$$\text{s.t. :} \left(\begin{array}{lll} \max\limits_{v_j(t)} & v^{growth}(t) & \forall \quad j \in Q \\ \text{s.t. :} & \sum\limits_{j=1}^{M} S_{kj} v_j(t) = 0 & \forall \quad k \in N \\ & \alpha_j \leq v_j(t) \leq \beta_j & \forall \quad j \in M \\ & v_j(t) = v_c^- & \forall \quad j \in U,\, t < t_{reg} \\ & v_j(t) = v_c^+ & \forall \quad j \in U,\, t \geq t_{reg} \\ & v_{pts}(t) + v_{glk}(t) = v_{glucose}^{uptake}(t) & \end{array} \right)$$

$$\begin{aligned} t_f &= 12\text{h} \\ \mathrm{d}P/\mathrm{d}t &= v^{product}(t)X \\ \mathrm{d}X/\mathrm{d}t &= v^{growth}(t)X \\ \mathrm{d}G/\mathrm{d}t &= -v_{glucose}^{uptake}(t)X \end{aligned}$$

The estimate of the unknown model parameter is updated using a scheme related to the Model-Reference Adaptive Systems (MRAS) [18]. The update of the parameter estimate with each successive batch is given as:

$$\theta(j+1) = \theta(j) + \gamma(j) e(j) \left[\frac{\partial e(j)}{\partial \theta(j)} \right]^{-1} \tag{4}$$

where θ represents the unknown model parameter, e represents the model error ($e = y^{actual} - y^{model}$), j represents the batch number and γ represents the adaptation rate determined by the following rule:

$$\begin{array}{lll} \gamma(j) = 5\gamma(j-1) & \text{if} & \left[\begin{array}{l} \frac{|e(j)-e(j-1)|}{e(j-1)} < 0.1 \\ \text{\&}\ |e(j)| > 0.5 \end{array} \right] \\ \gamma(j) = \gamma(j-1)/2 & \text{if} & |e(j)| > 1.25|e(j-1)| \\ \gamma(j) = \gamma(j-1) & & \text{otherwise} \\ \gamma(0) = 0.025 & & \end{array}$$

The choice of the adaptation rate is crucial for error and parameter convergence. The optimal value of the adaptation rate is difficult to estimate due to the nonlinear rate of error change with change in parameter and the unknown measurement error [18]. In this work, a heuristic approach is developed for dynamically determining the adaptation rate. The initial value is chosen such that the convergence is slow. The optimal genetic alteration is predicted using the initial guess of the unknown parameter by the bilevel optimization of Equation (3). The error between the 'actual' system and the model prediction is obtained. This error is used to correct the parameter estimate using Equation (4) and the procedure is repeated for the next batch with the new parameter estimate. With each iteration, the adaptation rate is updated to compensate for slow or fast convergence. At each stage a convergence test is performed to determine whether the correct parameter has been obtained. If the estimated parameter value does not change with each successive iteration then convergence is assumed.

The bilevel optimization problem is solved in MATLAB. The outer nonlinear optimization is evaluated using $fmincon$; whereas, the inner linear optimization is evaluated using $linprog$. In case of the nonlinear optimization, the evaluation is performed from multiple initial conditions to approximate the globally optimal result.

IV. RESULTS

A. Case study I: Batch control with high batch time penalty

In this section, the effect of the penalty for longer batch times on the optimal genetic regulation strategy is determined. The penalty is imposed by the weight (W) in (1). Batch times higher than the base value (t_{base}) are penalized. This base time is taken to be 10 h. Further, the final batch time is constrained by an upper bound of 15 h. The weight is increased from zero (no penalty) to higher values where the penalty is severe to reduce the batch time to the base value of 10 h. For each choice of weight the bilevel optimization of (1) is solved to determine optimal regulation time for the acetate flux and the batch time.

Fig. 3 shows the results for the batch fermentation where there was no inhibition to growth due to high ethanol concentrations. In this case the glucose uptake rate was fixed at 10 mmol g-biomass^{-1} h^{-1}. It is observed that for very low weights ($W \leq 0.1$), the penalty is not significant and the batch times are increased to the maximum value of 15 h. For these cases the increase in ethanol concentration with longer batch times was greater than the excess batch time penalty. The optimal regulation time was approximately 0.75 h. The early repression of the acetate flux results in

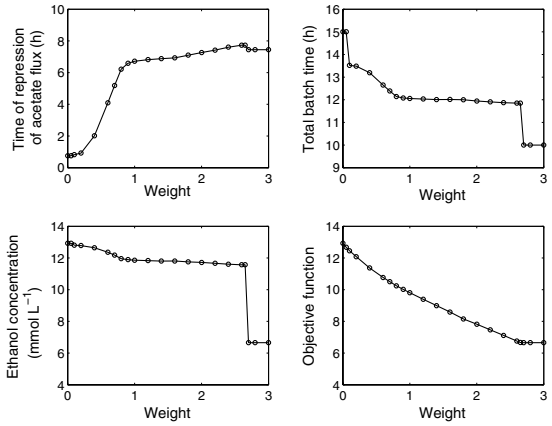

Fig. 3. Variations in the optimal regulation time of the acetate flux, the optimal batch time, the final ethanol conc. and the objective function value with changes in the weight penalty for batch anaerobic growth of $E.\ coli$ under no growth inhibition

reduced growth rates during the batch fermentation, but a higher ethanol flux. The growth rates are affected but the longer batch time allow for the complete consumption of the glucose. Thus, it is preferable to operate with an inactive acetate flux leading to increased final ethanol concentration of around 12.9 mmol L^{-1}. An increase in the weight above 0.1 results in a drop in the batch time to 13.5 h. This decrease in batch time reduces the penalty in the objective function. The batch time drops to around 12 h with the weight increase to 1. This is associated with an increase in the optimal repression time of the acetate flux from 0.8 h to 6.7 h. This is expected because for shorter batch times, it is preferable to have an active acetate flux for a longer period such that all the glucose is optimally consumed. Over this weight change the ethanol concentration decreases from 12.9 mmol L^{-1} to 11.8 mmol L^{-1}. With a further increase in the weight from 1 to 2.65 there is a gradual drop in the batch time from 12 h to 11.85 h. There is an increase in the optimal repression time to 7.7 h and drop in the final ethanol concentration to 11.57 mmol L^{-1}. Over this range of the weight, the optimal genetic alteration solution is fairly independent of the weight. One explanation is that in this operating region a large decrease in the final batch time leads to a significant decrease in the ethanol concentration, and it is optimal to incur the penalty rather than decrease the ethanol production. This is observed by the constant decrease in the objective function value. For weights excess of 2.65, the penalty of high batch times exceeds the ethanol production and there is a drop in the batch time to the base

value of 10 h. For these cases the optimal regulation time is 7.45 h resulting in an ethanol production of only 6.65 mmol L^{-1}. Any further increase in weight does not alter the optimal solution.

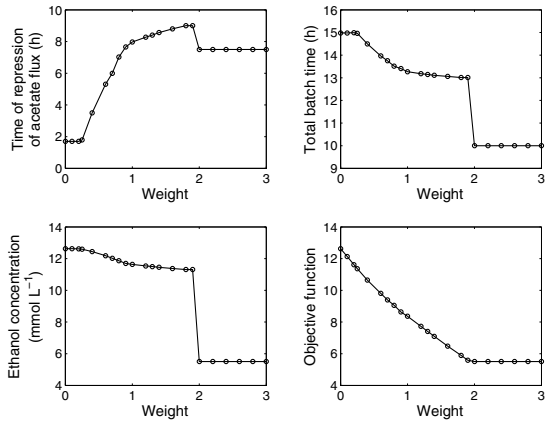

Fig. 4. Variations in the optimal regulation time of the acetate flux, the optimal batch time, the final ethanol concentrations and the objective function value with changes in the weight penalty for batch anaerobic growth of $E.\ coli$ under growth inhibition with increasing ethanol conc.

Fig. 4 shows the results for the batch fermentation in the presence of inhibition to the growth rate due to high ethanol concentrations. It is observed that the trends of the variations in the optimal genetic regulation follow that in the case where there was no growth inhibition. However, in the presence of inhibition the glucose uptake rates are effected during the course of the batch. This effect leads to subtle differences in the two cases. In this case the high batch time of 15 h is maintained up to a higher weight of 0.25 compared to 0.1 in the previous case. With a further increase in the weight the optimal batch time starts to decrease and the regulation time starts increasing as observed in the previous case. However, the optimal batch times are higher by about 1 h compared to the case with no inhibition. The reason for this is that due to lower glucose uptake rates longer batch times are required. Also, the optimal regulation times are higher resulting in an active acetate flux for longer periods. This has a dual effect, first, higher growth rates are maintained for an increased period to utilize all the glucose and second, inhibition to growth is reduced by preventing ethanol accumulation during the early periods of the batch. For the range of weights from 0 to 1.9, the ethanol concentration drops from 12.6 mmol L^{-1} to 11.3 mmol L^{-1}. For weights in the excess of 1.9 the batch time is reduced to the base value. Compared to the previous case, this occurs at a lower weight penalty. The reason for this is that ethanol production is lower than in the previous case due to growth inhibition and the high batch time penalty exceeds the ethanol production at a smaller weight. In both cases the drop occurs with the ethanol concentration around 11.5 mmol L^{-1} although this happens at different values of the weight W.

B. Case study II: Batch control under unmodeled system dynamics

The bilevel optimization framework provides the optimal genetic alteration profiles. However, the predictions are inaccurate in the presence of unmodeled system dynamics introduced as a result of unknown parameter values. In this section, the bilevel optimization framework is coupled with parameter estimation. With each batch run the unknown parameter is updated and bilevel solution is corrected. Batch fermentation in the presence of growth inhibition by high ethanol concentrations is considered. The inhibition constant is taken to be 25 mmol L^{-1} but is unknown to the control algorithm. Fig. 5 shows the variations of the optimal regulation time obtained by the bilevel optimization as a function of the inhibition constant. Asymmetric profiles in either direction of the actual value reflect the system nonlinearity. The final ethanol concentration at the end of the batch is used as the measurement for correcting the parameter estimates. To further test the robustness of the control algorithm, up to 5% measurement noise is added to the ethanol concentration measurement.

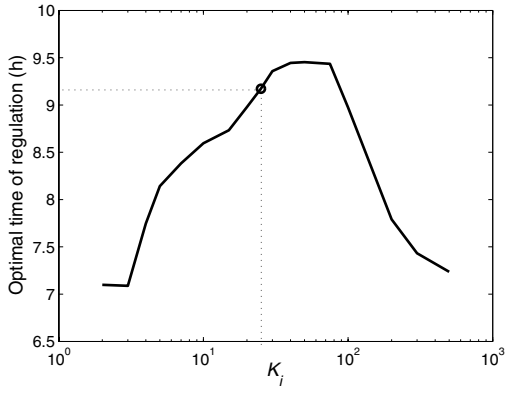

Fig. 5. Variations of the optimal time of regulation of the acetate flux as a function of the inhibition constant. The circle indicates the optimal regulation time for K_i=25 mmol L^{-1} (considered as the 'actual' system)

Two simulations are performed, one starting with a high initial guess of the unknown parameter ($K_i = 500$ mmol L^{-1}) and the second with a low initial guess ($K_i = 2$

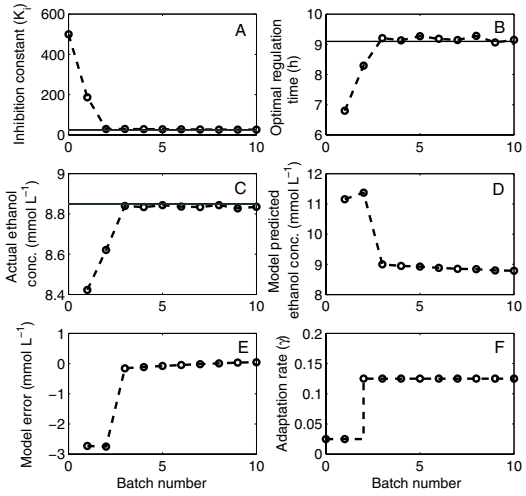

Fig. 6. A: Estimate of the inhibition constant starting with an initial guess of 500 mmol L^{-1}. The solid line indicates the inhibition constant for the 'actual' system. B: Optimal regulation time predicted by the bilevel optimization for the inhibition constant estimated after the previous batch run. The solid line indicates the optimal regulation time corresponding to the inhibition constant of 25 mmol L^{-1}. C: The final ethanol conc. for the 'actual' system with the regulation time corresponding to B. The solid line indicates the maximum ethanol conc. achievable. D: The final ethanol conc. predicted by the model using the estimated inhibition constant. E: Error in the model prediction for the ethanol conc. F: Adaptation rate

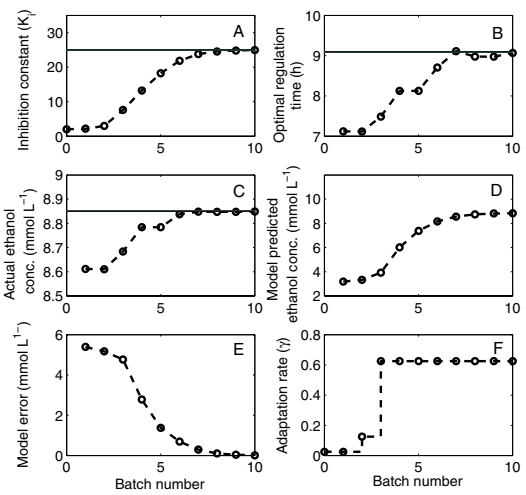

Fig. 7. A: Estimate of the inhibition constant starting with an initial guess of 2 mmol L^{-1}. The solid line indicates the inhibition constant for the 'actual' system. B: Optimal regulation time predicted by the bilevel optimization for the inhibition constant estimated after the previous batch run. The solid line indicates the optimal regulation time corresponding to the inhibition constant of 25 mmol L^{-1}. C: The final ethanol conc. for the 'actual' system with the regulation time corresponding to B. The solid line indicates the maximum ethanol conc. achievable. D: The final ethanol conc. predicted by the model using the estimated inhibition constant. E: Error in the model prediction for the ethanol conc. F: Adaptation rate

mmol L^{-1}). The two extreme values cover the entire range possible for the parameter value. In each case the control algorithm scheme shown in Fig. 2 is followed. Fig. 6 shows the results for the case with an high initial guess. It is observed that with an inhibition constant of 500 mmol L^{-1} the prediction of the optimal regulation time is around 7 h which results in an ethanol production of 8.4 mmol L^{-1}. The error in the model prediction and the actual system is -2.75 mmol L^{-1}. This error is used to update the estimate of the inhibition constant and the process is repeated. Within two iterations an estimate of 29.6 mmol L^{-1} for the unknown parameter is obtained. With this parameter value an optimal regulation time of 9.2 h and an ethanol concentration of 8.84 mmol L^{-1} is obtained. This is the maximum ethanol concentration achievable. With further iterations operation is maintained at the optimal solution.

Fig. 7 shows the results starting with an initial guess of 2 mmol L^{-1} for the unknown parameter. The initial optimal regulation time is predicted around 7.1 h resulting in a suboptimal ethanol production. However, the parameter value is corrected with each iteration and within 4 iterations the ethanol production comes within 1% of the maximum value. Within 7 iterations the parameter estimate converges to the correct value. The optimal conditions are maintained with subsequent iterations. Compared to the previous case the convergence is slower for this case, due to smaller sensitivity of the model error to parametric changes at small parameter values. The convergence is prevented from being excessively sluggish by the increase in the adaptation rate.

V. CONCLUSIONS

In this work, the bilevel optimization framework developed previously [1] was coupled with batch control algorithms to maximize the ethanol concentration at the end of the batch in an anaerobic fermentation of $Escherichia\ coli$. The first case study demonstrated the dependence of the optimal genetic alterations strategies on operational penalty of excess batch time. The optimal time of regulating the acetate flux varied considerably for both cases of growth; with and without inhibition due to high ethanol concentrations. The bilevel optimization was successfully augmented to incorporate the excess batch time penalty and both the optimal genetic manipulation and the optimal batch times were determined.

The second case study involved determining optimal genetic manipulations in the presence of unmodeled dynamics of the growth inhibition by high ethanol concentrations. The bilevel optimization framework was coupled to a parameter estimation algorithm. With each iteration the estimate of the unknown parameter was corrected with the measurements obtained at the end of the previous batch run. With an improvement in parameter estimates the ethanol production achieved is greater than 99% of the maximum value within 4 iterations starting from extreme initial guess of the unknown inhibition constant. Here, the utility of framework was demonstrated for the inhibition constant as the unknown parameter. The framework can be easily extended to other model parameters as well as multiple unknown parameters.

In this work, it was demonstrated that the bilevel optimization framework to determine optimal genetic alteration profiles could be easily extended to incorporate additional operation requirements. Further, the framework could be coupled with batch control algorithms to perform optimally in the presence of plant-model mismatch and measurement errors. The framework thus shows tremendous promise to provide dynamic strategies for metabolic engineering in practical operating situations.

NOMENCLATURE

e	model error
E	ethanol concentration (mmol L^{-1})
G	glucose concentration(mmol L^{-1})
K_i	inhibition constant
M	total number of fluxes
N	total number of metabolites
P	product concentration (mmol L^{-1})
Q	set of all unregulated fluxes
S	stoichiometric matrix
t_0	initial time (h)
t_f	final batch time (h)
t_f^{max}	upper bound on batch time (15 h)
t_{reg}	optimal regulation time (h)
U	set of regulated fluxes
v	flux (mmol g-biomass^{-1} h^{-1})
v_c^-	manipulated flux value for $t < t_{reg}$
v_c^+	manipulated flux value for $t \geq t_{reg}$
$v_{glucose}^{uptake}$	maximum glucose uptake flux in the presence of growth inhibition
$v_{glucose}^{uptake\,*}$	maximum glucose uptake flux in the absence of growth inhibition
v^{growth}	growth rate (h^{-1})
$v^{product}$	product flux (mmol g-biomass^{-1} h^{-1})
X	biomass concentration (g L^{-1})
α_j	lower bound of flux j
β_j	upper bound of flux j
γ	adaptation rate
θ	unknown model parameter

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