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# Design and Evaluation of an Optimal Controller for Simultaneous Saccharification and Fermentation Process

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**Abstract** Ethanol from corn is produced using dry grind corn process in which simultaneous saccharification and fermentation (SSF) is one of the most critical unit operations. In this work an optimal controller based on a previously validated SSF model was developed by formulating the SSF process as a Bolza problem and using gradient descent methods. Validation experiments were performed to evaluate the performance of optimal controller under different process disturbances that are likely to occur in practice. Use of optimal control algorithm for the SSF process resulted in lower peak glucose concentration, similar ethanol yields (13.38±0.36% v/v and 13.50±0.15% v/v for optimally controlled and baseline experiments, respectively). Optimal controller improved final ethanol concentrations as compared to process without optimal controller under conditions of temperature (13.35±1.28 and 12.52±1.19% v/v for optimal and no optimal control, respectively) and pH disturbances (12.65±0.74 and 11.86±0.49% v/v for optimal and no optimal control, respectively). Cost savings due to lower enzyme usage and reduced cooling require-

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ment were estimated to be up to $1 million for a 151 million L/yr (40 million gal/yr) dry grind plant.

**Keywords** Dry grind corn ethanol · *Saccharomyces cerevisiae* · Cybernetic model · SSF process · Optimal controller · Gradient descent · Process disturbances

## Introduction

Ethanol from corn is mostly produced using dry grind corn process in which simultaneous saccharification and fermentation (SSF) is one of the most critical unit operations. Temperature, pH and the glucoamylase dose are three variable that could be controlled in SSF process. A simplified control strategy for SSF process control is generally followed in most dry grind plants. Mash temperature is maintained at 30°C throughout the SSF process; whereas, pH is adjusted prior to SSF and not controlled thereafter. Glucoamylase enzyme is dosed within the first 10 h of SSF process in two/three steps. SSF reactor temperature and pH are monitored in most dry grind corn plants. Complexity of the fermentation process poses a challenge for development of a reliable, optimal controller that can improve SSF performance under varying conditions of pH, temperature and yeast growth. Hence, most commercial plant adopt the above mentioned simple, yet reliable fixed set point strategy to minimize the risk of inefficient SSF process. However, some difficulties in using such a control strategy exist in practice. For example, in hot weather cooling capacity of the plant is reached and the set point temperatures are not maintained resulting in SSF reactors operating at higher temperatures for some time during the SSF process. Similar issues also exist during extreme cold weather where the SSF process temperature is lower than the set point temperature. Another example is that manufacturer's reported minimum activity level is often used for determining glucoamylase dose, while in reality the actual activity of this enzyme is generally higher. However, due to storage and other factors, the glucoamylase activity decreases with time and is difficult to measure activity on a daily/batch-batch basis in industrial setting. Therefore all the enzyme dosings are based on the manufacturer's reported minimum activities. Similarly, SSF reactor pH is influenced by amount of recycled thin stillage (backset), buffering capacity of the mash and bacterial contamination. Thus control of fermenter temperature, pH and glucoamylase dose using a constant set point control approach may not achieve optimum performance. Additionally, since operating variables are not changed in response to fermenter conditions, optimal performance (minimize operating costs such as heating, cooling, use of chemicals and enzymes, while maintaining or achieving higher final ethanol concentrations) may not be achieved under all conditions.

Under normal circumstances high overall SSF process efficiencies of 90% are generally achieved in industrial scale fermenters, thus there is little scope for increasing the overall efficiencies of the process by application of a control strategy. On the other hand there is a potential for development of optimal control strategies that can reduce enzyme and energy use during SSF process that use existing plant controls with minimum additional capital cost. Such a controller would have to be reliable and capable of handling undesired fluctuations in temperature, pH and constantly adapt to any changes in the glucoamylase activity.

Control of the fermentation process should reduce chemicals (enzymes, acid/alkali) and utilities (cooling water) requirement and minimize fermentation time by continuously responding to fermenter conditions. Improving fermenter performance by incorporating a controller could also increase ethanol yield by minimizing residual starch and glucose fractions. An optimal controller could be used to determine set point profiles for fermenter temperature, pH and amount of glucoamylase to achieve such an optimum performance. As opposed to 'static' set point controllers, the set point profiles for temperature, pH and glucoamylase amount could change in response to varying operating conditions, with optimal controller.

Control of bioreactors has been investigated using several approaches by many researchers [2, 4, 5, 7–10, 15–17, 21, 23, 24]. Most have used fed batch or continuous fermentations, where the feed rate to the fermenter was controlled continuously. Fed batch fermentations operate in steady state conditions and controllers maintain the system in a desirable steady state. Disturbances perturbing the system from optimal states are controlled by manipulating nutrient feed rate, fermenter temperature, mash pH and dissolved oxygen concentration.

In the majority of models, researchers do not consider all dynamics of cellular metabolism. Further, even these models are linearized and systems are represented as linear time invariant models. Szederkènyi et al. [23] found that nonlinear models, even in the case of simple bioreactor models, have differences in terms of stability, time domain performance and model parameter tuning. Adaptive controls have been used for fed batch fermentations and are suitable for systems where process parameters are time varying. Chen et al. [3] developed a nonlinear adaptive controller for fed batch fermentation of glucose for ethanol production. They found the approach could stabilize an unstable process but may not optimize the process. Cybernetic modeling incorporates microorganism regulatory mechanisms and is better suited to applications where all measurements may not be available. Efforts by researchers [6, 19, 26] in the use of cybernetic models were limited to yeast metabolism modeling. A cybernetic model structure, a set of coupled nonlinear differential equations, is amenable to controller design.

The SSF process in the dry grind corn process is affected by temperature, pH and bacterial infections. Using a previously validated SSF model based on cybernetic principles for yeast modeling, an optimal controller based on iterative gradient descent algorithm was developed. Performance of the optimal controller was tested under externally applied disturbances during the SSF process.

Specific objectives of this research were to:

1. Develop an algorithm to optimize the SSF process using an optimal controller for the SSF model.
2. Test the optimal controller performance during the SSF process under:
   (a) normal operating conditions,
   (b) temperature disturbance,
   (c) pH fluctuation.
3. Compare glucoamylase use in SSF process with and without the optimal controller.

## Design of Optimal Controller

A model that captures the dynamics of the SSF process is a prerequisite for the design of a controller. A previously validated model for simultaneous saccharification and fermentation (SSF) model, outlined in Appendix: A, was used as the basic framework for controller design [11–13]. Using cybernetic principles, a model of the yeast was developed to incorporate the effects of process variables such as temperature, pH, enzyme dosage, initial inoculum, substrate and acetic acid concentrations.

The model consists of a simplified enzymatic hydrolysis (saccharification) reaction. Substrate limitation or temperature effect on the enzymes was not considered relevant for the present problem as the substrate limitation will be active only towards the end of the fermentation and temperature range (28–40°C) does not significantly affect the enzyme activity. The fermentation model is an abstraction of the actual metabolic processes in glucose limited cultures of yeast (Fig. 1). The yeast cell is simplified into four enzymatic reactions for: aerobic and anaerobic reactions producing energy and redox metabolites ($EM$) such as ATP and NADH/NADPH; Conversion of glucose into structural metabolites ($SM$) that are used to make the components of daughter cells; and reaction for cell production from energy and structural metabolites. Since the yeast cell has limited intracellular material and energy resources, they regulate these reactions to optimize growth. This regulation of resource allocation with alternatives can be captured using the cybernetic modeling framework [22]. According to the matching law for resource allocation in cybernetic modeling, given a choice of alternatives a consumer allocates resources in conjunction with resources already invested to achieve maximum returns. The biological equivalent of resource allocation is synthesis of new enzymes while preferential utilization of past resources is similar to selective activation/deactivation of enzymes already present. The cybernetic variables $\nu$ and $u$ in Appendix: A govern the regulation of enzyme activities and synthesis for different pathways respectively.

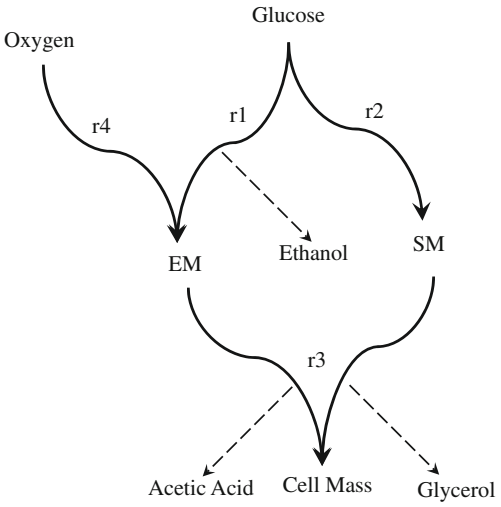


**Fig. 1** Proposed cybernetic model of yeast

This model (Appendix: A) was calibrated using experimental data to determine the parameters (Table 4) in the model [11]. This model was used to develop a controller to maximize final ethanol concentration in the fermenter while maintaining low glucose concentrations. Maximization of final ethanol concentration is the most important goal of the SSF process. Lower sugar concentrations during the SSF process reduce yeast osmotic stress and improve yeast cell viability. The controller is used to determine the set point values for the set point controller based on an optimal control algorithm. Since the set point control profiles are recalculated whenever HPLC measurements are available, control values may not be constant throughout the process and change in response to operating conditions. Hence, the controller is not 'static' but rather 'dynamic' in nature.

The SSF system of equations (Eq. 10) and the associated control design problem was formulated as a Bolza problem [18]. In Bolza problem, a scalar cost function is minimized subject to the differential equality constraints, admissible control constraints, and initial and terminal state constraints. After simplification using variational calculus, Lagrange multipliers and Hamilton–Jacobi theory, an algorithm based on steepest descent techniques [18] was used to find iteratively a convergent optimal solution. The optimal solution values ($u(t)$, i.e temperature, pH and glucoamylase amount) were used by the set point controller to control the SSF process. The SSF model (Eq. 10) was reformulated using matrix notation to simplify the development of the dynamic controller: Let,

$$\left.\begin{array}{l}\text{System states } [x_i]_{i=1\ to\ 11} = \left[X\ G\ E\ O\ EM\ SM\ e_1\ e_2\ e_3\ e_4\ GP\right]^T \\ \text{Control variables } \left[u_j\right]_{j=1\ to\ 3} = \left[T\ pH\ GA\right]^T \\ \text{System dynamics } \left[f_i\right]_{i=1\ to\ 11} = \text{Right hand side of SSF model defined in Eq. 10}\end{array}\right\} \tag{1}$$

The system defined by Eq. 10 can be compactly represented using Eq. 1 as:

$$\dot{x}_i = f_i(x, u, t) \quad \text{where, } i = 1 \text{ to } 11 \tag{2}$$

The goal of optimum control is to:

1. Maximize $x_3$ at a given final time ($t_f$) (i.e., maximize final ethanol concentration).
2. Minimize deviation of $x_2$ from a set point ($x_2 = 20$) at all times (i.e., minimize the deviation of glucose concentration from 20 g/L. A glucose concentration of 20 g/L has been assumed to be the minimum level that does not affect adversely the SSF process).

Defining $J$, a cost function that comprises of process goals to be achieved throughout the process, the optimal controller design problem for Eq. 2 can be posed as follows.

Obtain an optimal control vector ($u(t)$) that minimizes the cost function ($J$):

$$J = \theta(x,t)|_{t_0}^{t_f} + \int_{t_0}^{t_f} \left\{\phi(x,u,t) + \lambda^T[f_i(x,u,t) - \dot{x}] + \left(U^T \mathrm{H}_{U,0}\right)\right\} dt \tag{3}$$

Subject to :

1. Differential system equality constraints: $\dot{x}_i = f_i\,(x, u, t)$ (System dynamics)
2. Initial condition equality constraints: $x(t_0) = x_0$

3. Admissible control vector constraints:

$$\left.\begin{array}{l} 20 \le u_1 \le 35 \ \ (^{\circ}\mathrm{C}, \text{temperature limits}) \\ 3.5 \le u_2 \le 5.0 \ \ (\text{pH limits}) \\ \dfrac{\mathrm{d}u_3}{\mathrm{d}t} \ge 0 \ \ (\text{enzyme dosage limits}) \end{array}\right\} \tag{4}$$

Inequality constraints can be changed to equality constraints as follows [18]:

$$\left.\begin{array}{l} \left(u_{1,max} - u_1\right)\left(u_1 - u_{1,min}\right) = \Gamma_1^{-2} \\ \left(u_{2,max} - u_2\right)\left(u_2 - u_{2,min}\right) = \Gamma_2^{-2} \\ \dfrac{\mathrm{d}u_3}{\mathrm{d}t} \ge 0 \Rightarrow u_{3,min} \le u_3 \le u_{3,max} \\ \left(u_{3,max} - u_3\right)\left(u_3 - u_{3,min}\right) = \Gamma_3^{-2} \end{array}\right\} \tag{5}$$

Where $\Gamma_1$, $\Gamma_2$ and $\Gamma_3$ are constants.

$$U = \begin{bmatrix} \Gamma_1^2\left(u_{1,max} - u_1\right)\left(u_1 - u_{1,min}\right) - 1 \\ \Gamma_2^2\left(u_{2,max} - u_2\right)\left(u_2 - u_{2,min}\right) - 1 \\ \Gamma_3^2\left(u_{3,max} - u_3\right)\left(u_3 - u_{3,min}\right) - 1 \end{bmatrix} \tag{6}$$

Define:

$$\text{Hamiltonian is defined as: } \mathscr{H} = \phi(x,u,t) + \lambda^T f(x,u,t) \tag{7}$$

In the cost function (Eq. 3), first term on the right hand side $(\theta(x,t))|_{t_0}^{t_f})$ defines the process goal that is to be achieved at the final time, subject to the initial state constraints. The first term $(\int_{t_0}^{t_f}\{\phi(x,u,t)\}\,dt)$ inside the integral defines the second goal (minimizing the deviation from the glucose set point) during SSF process. The second term inside the integral $(\int_{t_0}^{t_f}\{\lambda[f_i(x,u,t) - \dot{x}]\}\,dt)$ accounts for the system dynamic constraint. The parameter $\lambda$ is the Lagrange multiplier that converts vector constraints into a scalar quantity $\mathscr{H}$ and transforms a vector performance index (defined by $\phi(x,u,t)$) to a scalar minimization problem (defined in terms of $J$ maximization/minimization). The third term inside the integral $(\int_{t_0}^{t_f}\left\{\left(U^T \mathrm{H}_{U,0}\right)\right\}\,dt)$ determines the penalty function for deviations outside the allowed ranges for the control variables. The magnitude of the penalty function depends on constants $\Gamma_1$, $\Gamma_2$ and $\Gamma_3$ chosen while defining $U$ in Eq. 6. Based on a variational calculus approach [18], a choice of $\Delta u$ that reduces $J$ (i.e. $\Delta J \le 0$) can be obtained as:

$$\Delta u = -k(t)\left[\frac{\partial \mathscr{H}}{\partial u} - \frac{\partial\left(U^T \mathrm{H}_{U,0}\right)}{\partial u}\right] \ \text{ where, } k(t) \text{ is a parameter gain matrix.} \tag{8}$$

Absolute change in $u$ is determined by the parameter gain matrix ($k(t)$). Absolute values of different control variables, such as temperature, pH and glucoamylase amount are dependent on the sensitivity of the SSF process to changes in these variables. Hence, the values in $k(t)$ are chosen (based on prior trial and error using model predictions) to account for the differences in the sensitivity of the control

variables. In general, $k(t)$ is dependent on total fermentation time and is a function of time. In this model, it is assumed to be static, i.e., a constant matrix. Therefore for any scale up of the system, $k(t)$ values need to be determined. This choice of $\Delta u$ is guaranteed to minimize cost function $J$ without violating the constraints on the control vector $u$ [18]. Steepest descent algorithm [18] was used to iteratively calculate the changes in $u$ until the change in cost function ($J$) was less than a small number, $\epsilon (> 0)$.

## Model Implementation

All the derivatives in the optimal controller were discretized using a first order finite difference scheme. The optimal controller was implemented using C++ language. A set point controller was developed and implemented using C++ to execute the temperature, pH and enzyme set points specified by the optimal controller. During various treatments, optimal controller algorithm was implemented after every 2 h mash sampling and HPLC analysis of the sample. Actual temperature, pH and enzyme dosage profiles were input as text files to the program. Generated output consisted of concentration profiles of new set point time series for temperature, pH and enzyme dosage. Some of the system states were measured directly using a HPLC method (glucose, ethanol, acetic acid and glycerol). Concentration of dextrins in the media was estimated based on a previously developed and validated stochastic liquefaction and saccharification model [11, 14]. Yeast biomass was estimated using a simplified yeast metabolic network as described in Murthy [11].

A computer controlled fermenter system was designed for testing the optimal controller. The control system architecture is shown in Fig. 2. This control architecture was implemented using a custom built fermentation system with set point controller for temperature, pH and glucoamylase enzyme (Fig. 3). Briefly, the system consisted

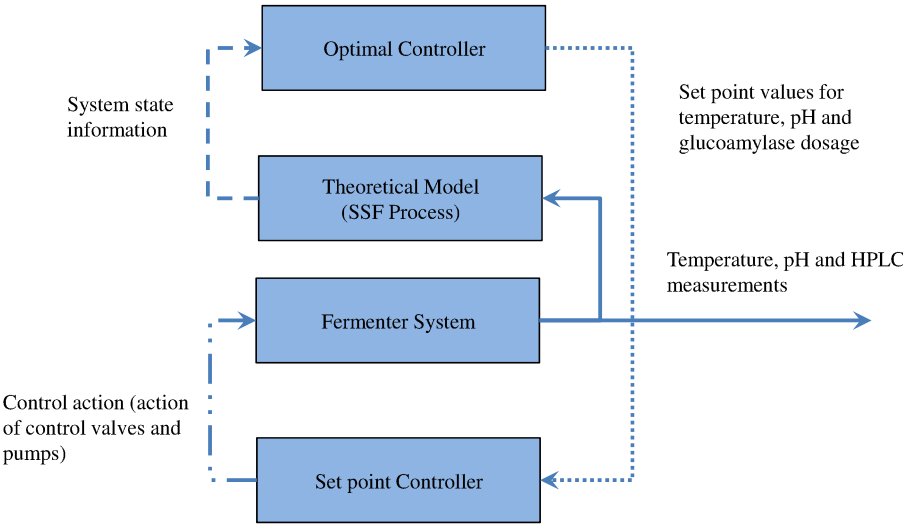


**Fig. 2** Control system architecture

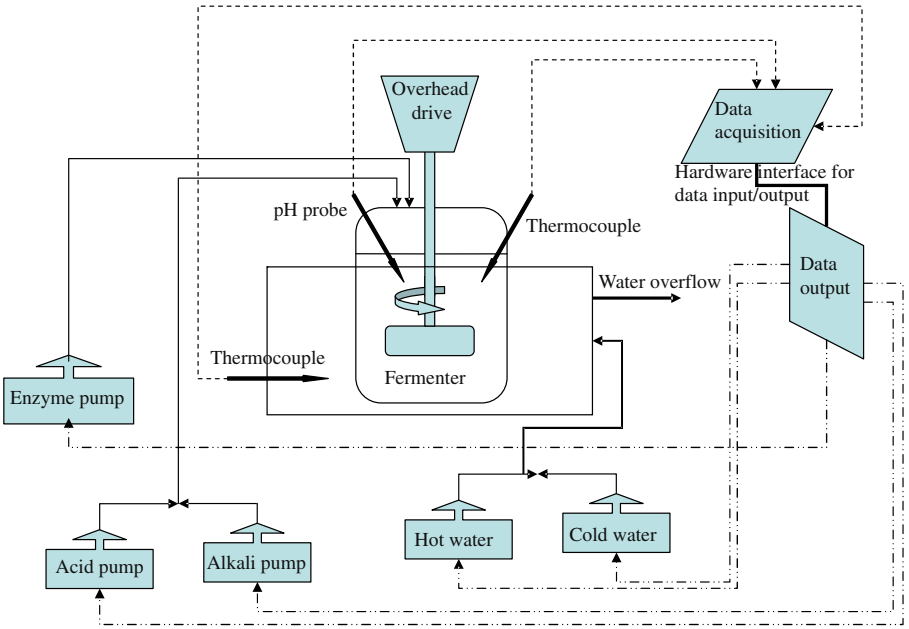


**Fig. 3** Schematic of the fermentation system to evaluate optimal controller

of a fermenter vessel (15 L, Bellco Glass, Vineland, NJ) housed in a water bath. Mash was agitated (100 rpm) using a paddle type agitator driven by a DC motor drive (model 7774-10000, Bellco Glass, Vineland, NJ).

Required heating or cooling was provided by circulating hot or cold water in the water bath. Uniform water bath temperature was achieved by recirculation of water in the water bath with a centrifugal pump (model AC-3C-MD, March Mfg., Glenview, IL). Temperature was measured using two thermocouples (T-type, Omega, Stamford, CT) placed in the fermenter and water bath. An ice box provided the reference junction temperature for the thermocouples. Solenoid valves (13A432, Dayton Electric, Niles, IL) on the hot and cold water lines were controlled by relays (W6210DSX-1, Magnecraft, Northfield, IL). Mash pH was measured using a pH probe (PHE 7352, Omega, Stamford, CT). Pumps required for pH control were a low volume positive displacement gear pump (PQM-1/115, Greylor, Capecord, FL) for alkali and a peristaltic pump (Masterflex 7535-10, Cole-Parmer, Chicago, IL) for acid. The pH controller activation of the appropriate pump was based on pH probe readings of mash pH. A high precision peristaltic pump (Masterflex L/S digital drive model 7523-50 and pump head model 7523-08, Cole-Parmer, Chicago, IL) was used to add required amounts of glucoamylase enzyme into the fermenter. The enzyme pump was set at a fixed volumetric flow rate and switched on using relays (W6210DSX-1, Magnecraft, Northfield, IL) for desired time duration. Data acquisition from thermocouples, pH probes and activation of relays for operation of acid/alkali pumps and hot/cold water lines was controlled by a data acquisition device (U12, Labjack, Lakewood, CO).

It was important to calibrate the system components and equipment such as thermocouples, pH probes, hammer mill and substrate characteristics to validate the developed models. The details of system components, testing and calibration are reported elsewhere [11].

## Optimal Controller Validation

Two sets of experiments were conducted to validate the optimal controller for the SSF process. In addition to the base case with/without optimal controller, experiments were conducted to evaluate the response to process perturbations likely to occur in industrial fermenters (Experiment 1). An additional set of experiments was conducted without optimal controller to compare the performance of SSF process to similar process fluctuations without optimal controller (Experiment 2). An overview of the validation experiments is presented in Table 1.

*Samples and Chemicals* Yellow dent corn (35D28, Pioneer Hi-Breed International, Johnston, IA) grown during the 2005 at the Agricultural and Biological Engineering Research Farm, University of Illinois at Urbana-Champaign was used. Samples were hand cleaned; kernel moisture content was determined using a convection oven method [1]. Proximate analysis, determined by near infrared spectrometer (model OmegaAnalyserG, Dickey-John, Springfield, IL), was 72.2% starch, 9.0% protein, 4.7% oil and 14.9% moisture (wb).

The $\alpha$-amylase (SpezymeFred, Genencor, Palo Alto, CA) and glucoamylase (Distillase L 400, Genencor, Palo Alto, CA) used in all experiments had activities of 21,390 activity units/mL and 315 amyloglucosidase units/mL, respectively. Urea, used as a nitrogenous nutrient for yeast, was obtained from Fischer (Fair Lawn, NJ). Active dry yeast (ADY) (Ethanol Red, Fermentis, Marcq-en-Baroeul, France) was used in all experiments. Mash pH was adjusted using concentrated $H_2SO_4$ (96% w/w) and NaOH (pellet form) obtained from Mallinckrodt Baker (Paris, KY) and

**Table 1** Outline of validation experiments

| Treatment | Treatment details |
|---|---|
| | Experiment 1: Perturbation with optimal control (15 L) |
| $E1T1_{Baseline}$ | Baseline (No optimal controller) |
| $E1T2_{OC}$ | Optimal controller without disturbances. |
| $E1T3_{OC,\ T}$ | A temperature disturbance (2 h, 20°C) from 24 to 26 h. |
| $E1T4_{OC,\ pH}$ | A pH disturbance (2 h, 2.8pH) from 24 to 26 h. |
| | Experiment 2: Perturbation without optimal control (200 mL) |
| $E2T1_{T,\ adjusted}$ | Temperature *adjusted* after temperature disturbance (2 h, 20°C) from 24 to 26 h. |
| $E2T2_{T,\ not\ adjusted}$ | Temperature *not adjusted* after temperature disturbance (20°C) at 24 h. |
| $E2T3_{pH,\ adjusted}$ | pH *adjusted* after an induced pH disturbance (2 h, 2.8pH) from 24 to 26 h. |
| $E2T4_{pH,\ not\ adjusted}$ | pH *not adjusted* after an induced pH disturbance (2.8pH) at 24 h. |

Sigma-Aldrich (St. Louis, MO), respectively. Tetracycline, an antibiotic, was obtained from Sigma-Aldrich (St. Louis, MO).

*Conventional Dry Grind Process* Hand cleaned corn samples (5,000 g) were ground in a pilot plant scale hammer mill (7" diameter, 3500 rpm, motor model G600 Allis-Chalmers, Nurwood, OH) equipped with a 0.5 mm round hole sieve. Moisture content of ground corn was determined using a convection oven method [1] prior to each experiment. Ground corn (3,750 g db) was mixed with hot water at 60°C to form 25% solids mash. Mash, liquefied using 3.75 mL $\alpha$-amylase for 90 min in a water bath maintained at 90°C, was cooled to 30°C and adjusted to 4.5 pH using $H_2SO_4$. Urea (1 g/L of mash) and ADY (0.7% w/w of starch) were added to the mash. Tetracycline was added at 6.67 ppm level to prevent bacterial contamination. As described below, liquefied mash was subjected to treatments using the experimental apparatus. Simultaneous saccharification and fermentation (SSF) was performed by adding 3.75 mL glucoamylase (manufacturer recommended dose) and 18.5 g of ADY. The SSF process was performed for 72 h with samples withdrawn every 2 h until 60 h.

*Experiment 1: Perturbation with Optimal Control*

All experiments were conducted with a 15 L mash volume containing 3,750 g initial dry solids (25%). Baseline experiments (Experiment 1, treatment 1; $E1T1_{Baseline}$), similar to the SSF process used in dry grind corn process, were conducted by a onetime initial addition of glucoamylase (0.1% w/w solids i.e., 3.75 mL) to the mash after addition of urea and ADY followed by adjustment to 4.5 pH. Temperature was maintained at 30°C during the SSF process.

Four treatments, in addition to baseline experiments were conducted to test effectiveness of the optimal controller. The second treatment (Experiment 1, treatment 2; $E1T2_{OC}$) consisted of evaluation of optimal controller without any externally induced disturbances.

In the third treatment (Experiment 1, treatment 3; $E1T3_{OC,\,T}$), a temperature disturbance was introduced randomly between 15 and 25 h and temperature was held at 20°C for 2 h. Under normal circumstances, temperature would be maintained at the required set point as set by operator/controller. However, temperature disturbances can occur in dry grind corn plants due to various factors such as improper mixing, failure of sensors and circulation pumps. Therefore, the plant is unable to maintain the temperature set by the controller/operators. These type of disturbances are very regular in structure (square/triangular wave type disturbances) and therefore a temperature disturbance was induced to simulate this case. Performance of optimal controller with a simulated temperature disturbance was evaluated.

The fourth treatment (Experiment 1, treatment 4; $E1T4_{OC,\,pH}$) consisted of a pH disturbance in which mash was changed to 2.8 pH and held at 2.8 pH from 24 to 26 h after start of the SSF process. Similar to temperature disturbances, pH disturbances can also occur in dry grind corn plants due to various factors such as bacterial infection, failure of alkali and acid pumps and pH may not be maintained at the required set point even after controller/operator intervention due to various types of failures. Performance of optimal controller with a simulated pH disturbance was evaluated.

Mash samples (2 mL) were drawn at 2 h intervals until 60 h and a final sample was drawn at 72 hr. Samples were analyzed using an HPLC method described below. Three replicate fermentations were conducted for all treatments including baseline experiments.

*Experiment 2: Perturbation without Optimal Control*

Five additional treatments were conducted on a smaller scale (50 g corn db, 25% solids wb) to evaluate the effect of the disturbances without the optimal controller. In a dry grind corn plant, correction measures could be initiated by manual temperature correction on detection of temperature disturbances. Such a scenario was simulated by adjusting the temperature of the fermenter back to 30°C after a disturbance (Experiment 2, treatment 1; $E2T1_{T,\ adjusted}$). In the second scenario the temperature was not adjusted to simulate breakdown of thermal regulation in the plant that could not be repaired during the batch time (Experiment 2, treatment 2; $E2T2_{T,\ not\ adjusted}$).

Similar to temperature disturbance correction in a dry grind corn plant, correction measures could be initiated by manual pH correction on detection of pH disturbances. Such a scenario was simulated by adjusting the pH of the fermenter back to 4.5 pH after externally induced disturbance (Experiment 2, treatment 3; $E2T3_{pH,\ adjusted}$). In the fourth scenario, the pH was left unchanged (Experiment 2, treatment 4; $E2T4_{pH,\ not\ adjusted}$). A control sample was processed using the SSF process along with the four treatments. Initial SSF (0 h) and final SSF (72 h) samples (2 mL) were drawn and processed using HPLC method described below. Three replicate fermentations were conducted for each treatment.

## HPLC Analysis

From each 2 mL sample, clear supernatant liquid was obtained by centrifuging the sample at 16,110×*g* (model 5415 D, Brinkmann-Eppendorf, Hamburg, Germany). Supernatant was passed through a 0.2 $\mu$m syringe filter into 150 $\mu$L vials. Filtered supernatant liquid (5 $\mu$L) was injected into an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) maintained at 50°C. Sugars (glucose, fructose, maltose and maltotriose), organic acids (lactic, succinic and acetic) and alcohols (ethanol, methanol and glycerol) were eluted from the column with HPLC grade water containing 5 mM $H_2SO_4$. Separated components were detected with a refractive index detector (model 2414, Waters Corporation, Milford, MA). Elution rate was 0.6 mL/min; a calibration standard ($DP4^+$ 0.44% w/v, maltotriose 0.5% w/v, maltose 2% w/v, glucose 2% w/v, fructose 1% w/v, succinic acid 0.5% w/v, lactic acid 1% w/v, glycerol 2% w/v, acetic acid 0.5% v/v, methanol 1% v/v and ethanol 20% v/v) was used to calibrate HPLC prior to each set of samples. Calibration standards were used as unknown secondary standards to check the consistency of the HPLC measurements. Data were processed using HPLC software (version 3.01, Waters, Milford, MA).

## Experimental Design

A complete randomized block design was used in each experiment. Each treatment was replicated three times. Each fermentation sample was analyzed using a mean of two HPLC injections. Analysis of variance and Tukey's test (SAS Institute, Cary,

NC) were used for mean separation at $\alpha = 0.4$. Economic analysis of potential cost savings from the process were calculated using glucoamylase enzyme price of \$0.006/L (\$0.024/gal) of ethanol [20].

## Results and Discussion

Performance of the optimal controller was evaluated by simulations and convergence of the algorithm. For an iterative algorithm it is essential to check the convergence criteria of the results because the number of iterations determines computer processor time. The need for shorter processor time is balanced with the need for convergence of control vectors. Balance can be achieved if the convergence criteria are set such that variation in the values of control vectors in subsequent iterations is less than the band of control variation achievable by the set point controller which controls the SSF process. For example, the SSF process was initiated with non-optimal initial values (temperature 20°C, 2.5 pH and glucoamylase 0.01 mL/L mash). The output of optimal control algorithm after one iteration was a constant temperature of 32.67°C (Fig. 4), a linearly decreasing pH from 4.5 to 3.3 (Fig. 5) and glucoamylase dose that increased linearly to 0.44 mL/L mash at 50 h and displayed an exponential rise thereafter (Fig. 6). When the SSF process was initialized with low glucoamylase dosage, corrective action of the optimal control algorithm caused the exponential rise in the glucoamylase dosage towards the end of the SSF process. This was the result of the primary goal of the optimal algorithm which was to maximize ethanol production by complete starch hydrolysis while maintaining favorable fermentation conditions.

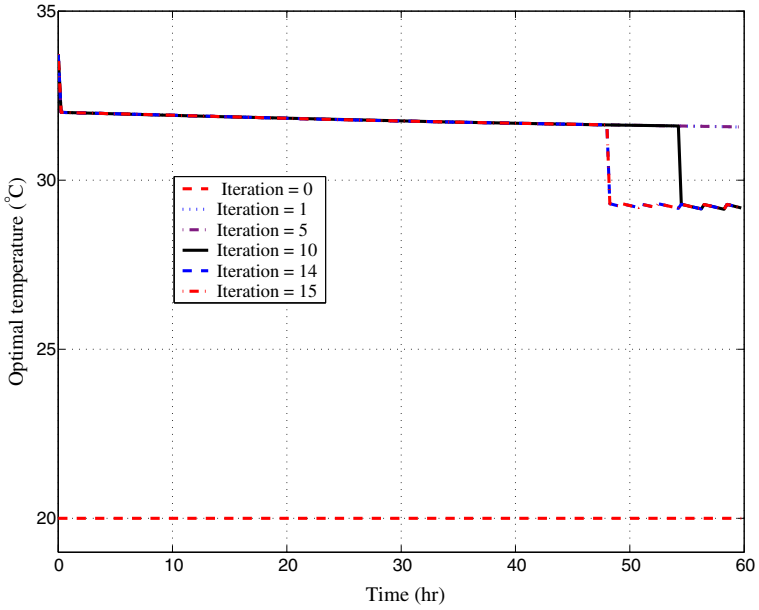


**Fig. 4** Predicted temperature profiles as a function of iteration number

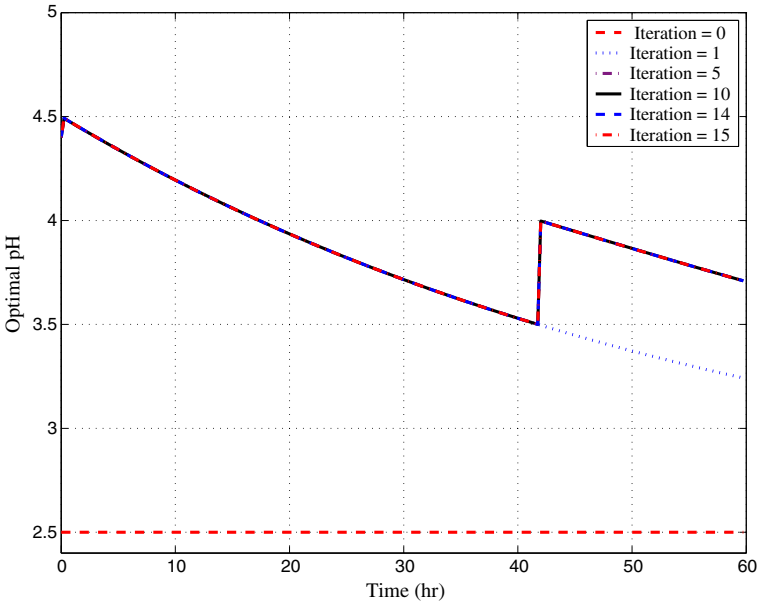


**Fig. 5** Predicted pH profiles as a function of iteration number

With increasing number of iterations (>10) the exponential increase disappeared and a more balanced profile was obtained (Fig. 6). Corresponding glucose and ethanol profiles are shown in Figs. 7 and 8, respectively. Final ethanol concentrations

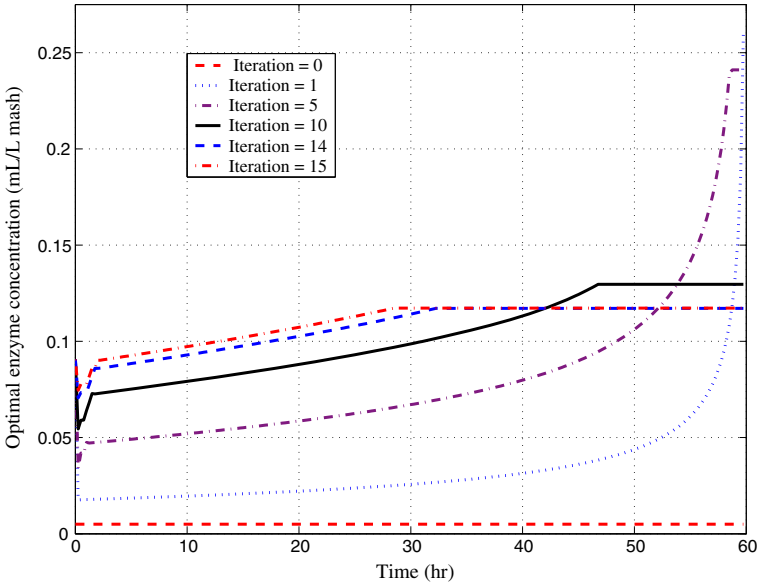


**Fig. 6** Predicted glucoamylase enzyme dose profiles as a function of iteration number

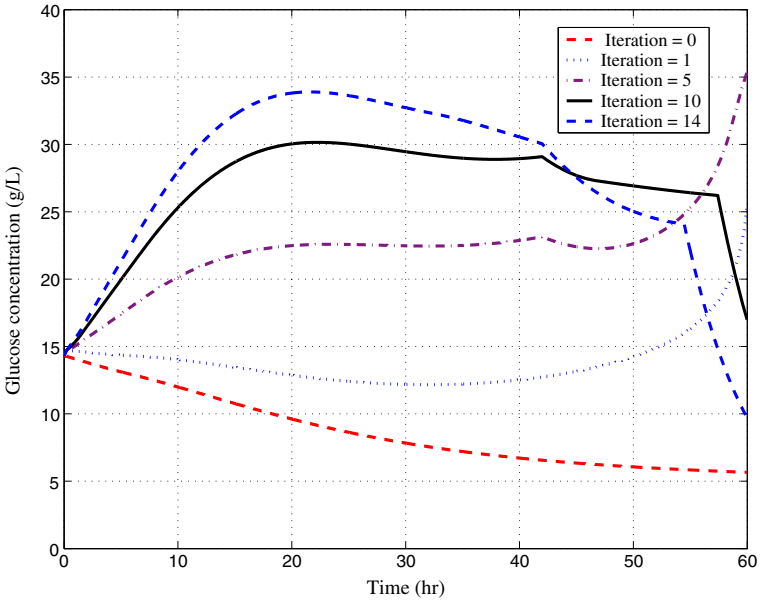


**Fig. 7** Predicted glucose concentrations (g/L) for different optimal profiles

increased with number of iterations as the set point control profiles were optimized progressively. Results obtained by varying the number of iterations indicate that after 15 iterations the variations in the control vector were very small and the best

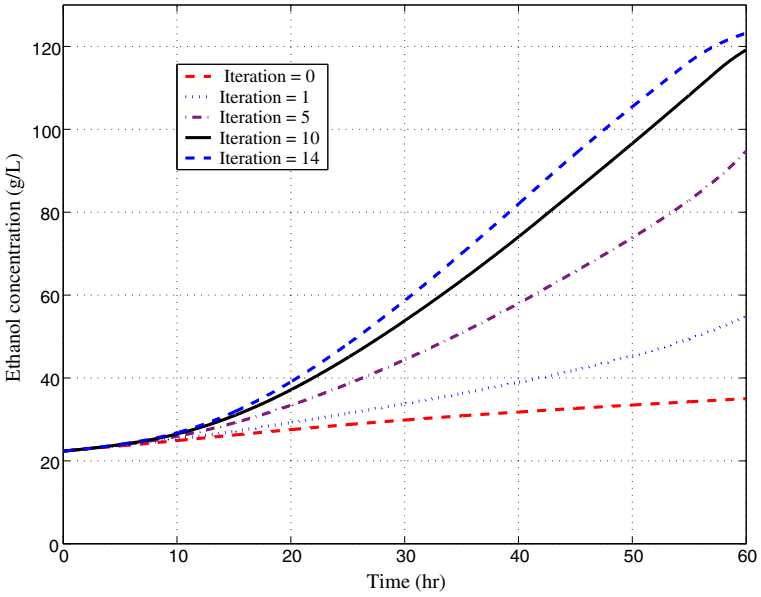


**Fig. 8** Predicted ethanol concentrations (g/L) for different optimal profiles

performance was achieved. After 15 iterations, the changes in the set point control profiles were too small to be controlled accurately by the set point controller. Hence the results obtained after 15 iterations of the optimal controller were used to control the SSF process. As compared to a conventional SSF process with no controller, there was potential for reduction in total glucoamylase enzyme requirement for an optimally controlled SSF process.

The set point temperature controller was able to maintain target temperature within ±0.5°C for a constant set point. However, for a variable temperature profile there were oscillations (±4°C) which were caused due to large inflow of hot or cold water into the waterbath on opening of hot or cold water valves, respectively. The pH probe showed a drift in the measured voltage over time and was not used in the experiments. Some of the possible reasons for the drift in pH probe included probe fouling and leaching of charged molecules from the mash into the probe. Therefore, instead of automatic pH adjustment using the pH probe and acid or alkali pumps, the pH was adjusted manually to the target pH values (determined by optimal control algorithm) every 2 h. The set point controller for control of glucoamylase enzyme dose was able to achieve a reliable and accurate tracking of the set points for glucoamylase dosage. Additional details on the performance of the set point controller are reported in [11].

Glucose concentrations in the optimally controlled SSF process ($E1T2_{OC}$) were lower than the baseline experiments ($E1T1_{Baseline}$) (Fig. 9). Lower glucose concentrations during SSF should have resulted in reduced osmotic stress to the yeast and thus improved yeast viability. However, ethanol concentration profiles

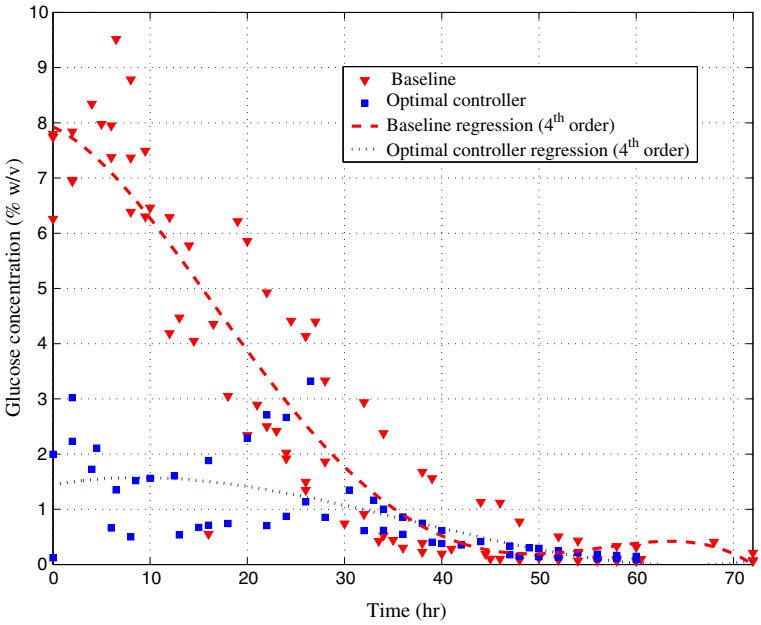


**Fig. 9** Comparison of glucose concentrations: optimal control vs baseline

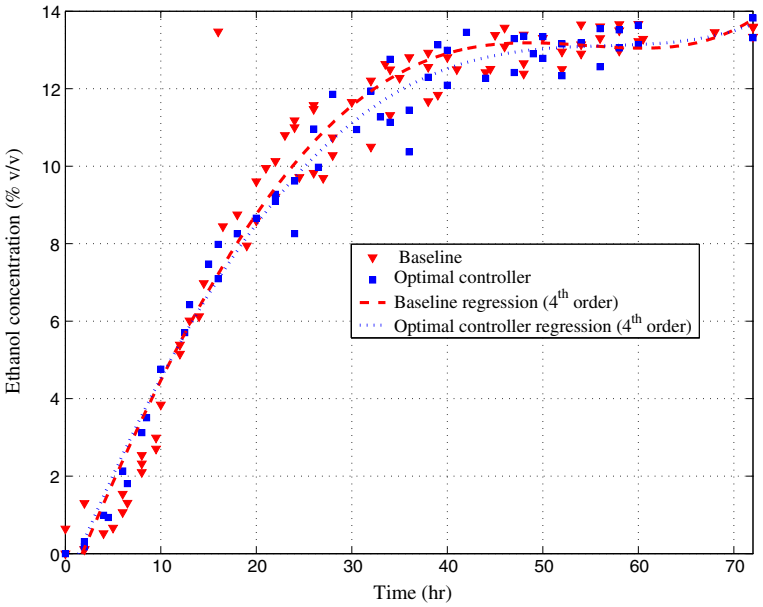


**Fig. 10** Comparison of ethanol concentrations: optimal control vs baseline

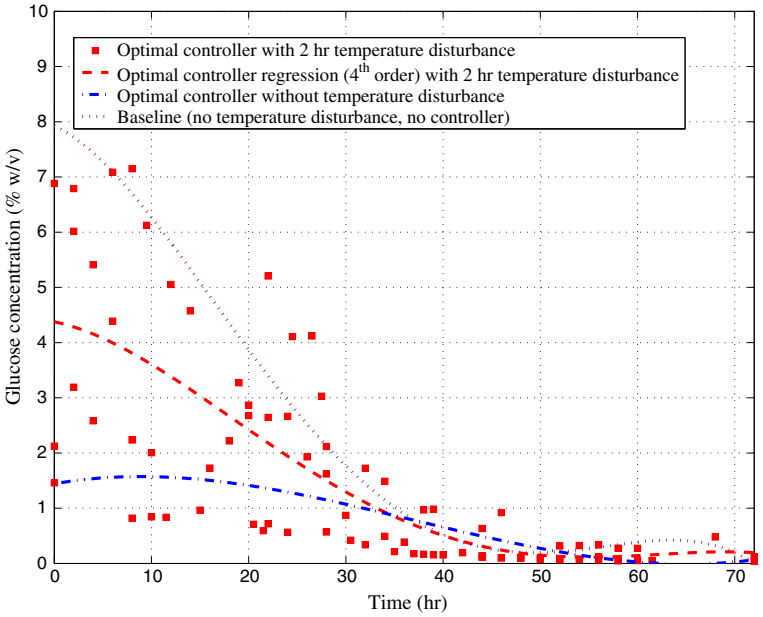


**Fig. 11** Comparison of glucose concentrations: temperature perturbation vs baseline

(Fig. 10) for $E1T2_{OC}$ were similar to $E1T1_{Baseline}$. Final ethanol concentrations for $E1T2_{OC}$ (13.38±0.36% v/v) were not statistically different from the $E1T1_{Baseline}$ (13.50±0.15% v/v). One of the significant results of this optimal controller implementation was that the amount of glucoamylase used in the $E1T2_{OC}$ was 1.86 mL; whereas, 3.75 mL (manufacturer recommended dose) was used in $E1T1_{Baseline}$ to achieve the same final ethanol concentrations. Yeast osmotic stress is more important in high gravity fermentations where the glucose concentrations are higher than 200 g/L during the SSF process. Hence use of optimal controller in high gravity fermentations can provide additional advantages.

Comparisons of glucose concentrations in the optimally controlled SSF process with a temperature disturbance ($E1T3_{OC,\,T}$) are shown in Fig. 11. Ethanol concentrations were lower for $E1T3_{OC,\,T}$ (13.35±1.28% v/v) and $E2T2_{T,\,not\,adjusted}$ (12.21±0.22% v/v) compared to $E1T1_{Baseline}$ (13.50±0.15% v/v)(Fig. 12). However, final ethanol concentrations for $E2T1_{T,\,adjusted}$ (12.52±1.19% v/v) and $E2T2_{T,\,not\,adjusted}$ (12.21±0.22% v/v) were significantly lower (0.83% v/v) than final ethanol concentration for $E1T3_{OC,\,T}$ (13.35±1.28% v/v) (Fig. 12). This indicates that use of optimal controller can result in increased final ethanol concentrations when SSF process is subject to temperature fluctuations.

Comparisons of glucose concentrations in the optimally controlled SSF process with a pH disturbance ($E1T4_{OC,\,pH}$) are shown in Fig. 13. As compared to baseline experiments ($E1T1_{Baseline}$), ethanol concentrations were lower for the SSF process with pH disturbance (Fig. 14). Final ethanol concentration was higher (12.65±0.74%

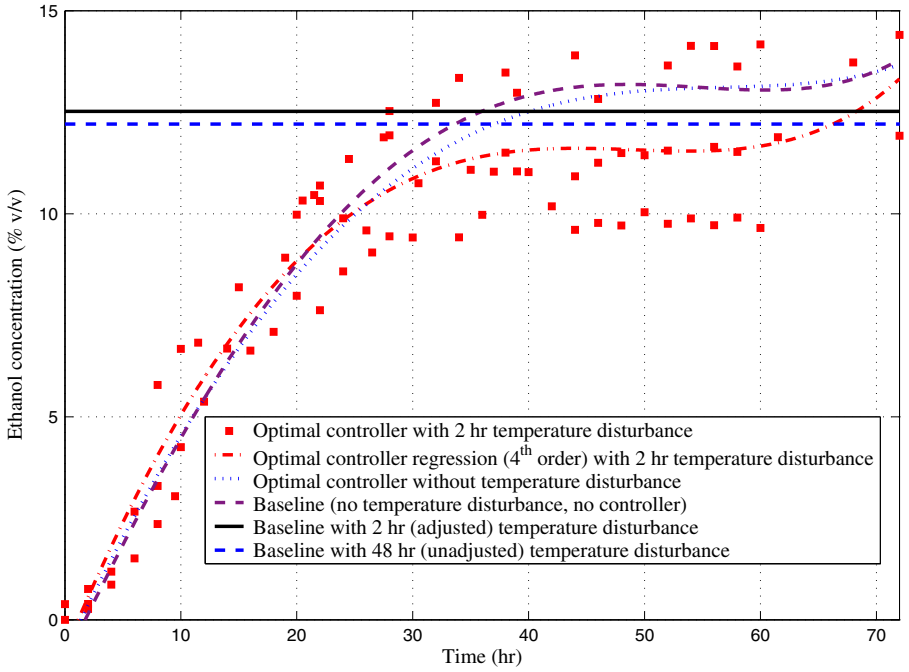


**Fig. 12** Comparison of ethanol concentrations: temperature perturbation vs baseline

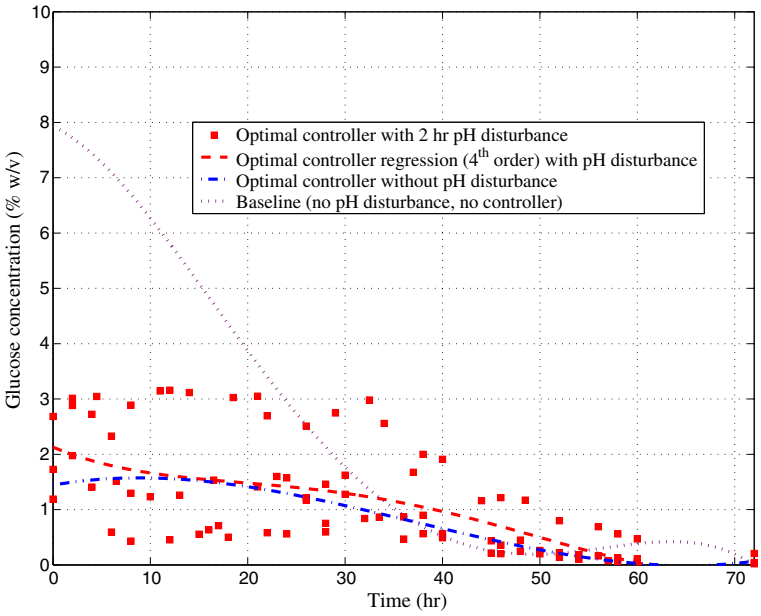


**Fig. 13** Comparison of glucose concentrations: pH perturbation vs baseline

v/v) for $E1T4_{OC,\,pH}$ compared to $E2T3_{pH,\,adjusted}$ (11.86±0.49% v/v). Similarly, final ethanol concentrations were also higher for $E1T4_{OC,\,pH}$ (12.65±0.74% v/v) compared to $E2T4_{pH,\,not\,adjusted}$ (12.44±0.33% v/v). Lower fermenter pH leads to increased maintenance requirements for the yeast cell and results in increased ethanol production [25]. Increased carbon flux through the fermentation pathway leads to lower cell mass yields. This phenomenon could have resulted in the observed significantly higher ethanol concentrations in $E2T4_{pH,\,not\,adjusted}$ (12.44 ±0.33% v/v) compared to $E2T3_{pH,\,adjusted}$ (11.86 ±0.49% v/v). However, even in this case, use of optimal controller resulted in higher final ethanol concentrations for SSF process with pH fluctuations. Final glucose and ethanol concentrations for all the validation experiments are summarized in Table 2.

Using optimal controller for SSF process, glucoamylase amount required in the SSF process was reduced 50% compared to baseline experiments. This reduction was observed even in the case of simulated process disturbaces in temperature and pH that were designed to mimic likely operational difficulties that a plant would face in maintaing set point temperatures and pH. Significantly in all such cases, final ethanol concentrations were significantly higher with the use of optimal controller for SSF process (Table 2). Assuming the cost of glucoamylase enzyme to be \$0.006/L (\$0.024/gal) of ethanol [20], a 50% reduction in glucoamylase usage could lead to \$480,000/yr savings in a 151 million L/yr (40 million gal/yr) ethanol plant. The SSF profiles using optimal control had on an average higher fermenting operating temperatures (32°C) compared to the baseline with no optimal control (30°C). Higher temperatures (32°C) maintained during the SSF process with optimal controller, compared to a constant process temperature (30°C) in a conventional

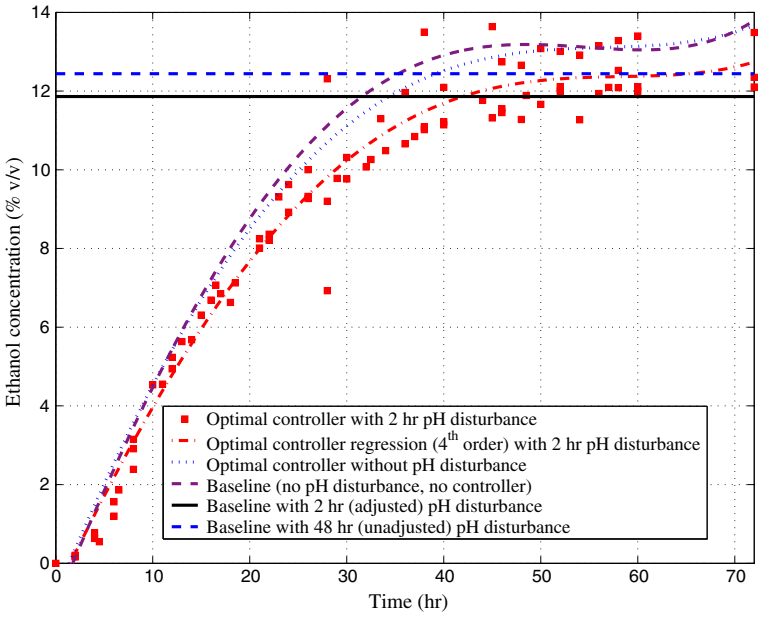


**Fig. 14** Comparison of ethanol concentrations: pH perturbation vs baseline

SSF process, required 7% less energy (as cooling water) and thus could result in potential reduction of cooling water requirements. Assuming an energy cost of \$0.057/L (\$0.2142/gal) of ethanol [20], reduced energy requirements could lead to \$600,000/yr savings (Table 3).

Use of the optimal controller could reduce the proliferation of bacteria by reducing the available glucose and thus result in reduced antibiotic requirements. Reduced antibiotic usage would decrease the concentration of antibiotics in DDGS and would increase the marketability of DDGS. Optimal controller use would improve fermentation characteristics and reduce the variability in DDGS composition

**Table 2** Final ethanol and glucose concentrations in validation experiments

| Treatment | Glucose (%w/v) | Ethanol (%v/v) | Glucoamylase (% w/w mash solids) |
|---|---|---|---|
| Experiment 1: Perturbation with optimal control (15 L) | | | |
| $E1T1_{Baseline}$ | $0.115 \pm 0.082$ | $13.50 \pm 0.15$ | 0.1 |
| $E1T2_{OC}$ | $0.134 \pm 0.04$ | $13.38 \pm 0.36$ | 0.05 |
| $E1T3_{OC,\ T}$ | $0.214 \pm 0.23$ | $13.35 \pm 1.28$ | 0.05 |
| $E1T4_{OC,\ pH}$ | $0.09 \pm 0.10$ | $12.65 \pm 0.74$ | 0.05 |
| Experiment 2: Perturbation without optimal control (200 mL) | | | |
| $E2T1_{T,\ adjusted}$ | $0.15 \pm 0.18$ | $12.52 \pm 1.19$ | 0.1 |
| $E2T2_{T,\ not\ adjusted}$ | $0.17 \pm 0.19$ | $12.21 \pm 0.22$ | 0.1 |
| $E2T3_{pH,\ adjusted}$ | $1.05 \pm 0.41$ | $11.86 \pm 0.49$ | 0.1 |
| $E2T4_{pH,\ not\ adjusted}$ | $0.754 \pm 0.59$ | $12.44 \pm 0.33$ | 0.1 |

**Table 3** Estimated savings with use of optimal controller assuming total enzyme and energy cost of \$0.0126/L (\$0.048/gal) and \$0.057/L (\$0.2142/gal), respectively [20]

| Plant capacity | Savings (×\$1000\$/yr) | | |
|---|---|---|---|
| (million L/yr (gal/yr)) | Enzyme | Energy | Total costs |
| 57 (15) | 180 | 227 | 407 |
| 151 (40) | 480 | 605 | 1,085 |
| 227 (60) | 720 | 908 | 1,628 |
| 284 (75) | 900 | 1,135 | 2,035 |
| 378 (100) | 1,200 | 1,513 | 2,713 |
| 568 (150) | 1,800 | 2,269 | 4,069 |

caused by variability in residual glucose concentrations at the end of SSF process. Reduced final glucose concentrations are expected to reduce the potential problems during drying (Millard reactions) and handling (caking) of DDGS. Maintaining lower glucose concentrations in the SSF process can lead to indirect benefits such as increased bioavailability of the lysine by reducing its reaction with reducing sugars during DDGS drying process and degradation to furosine [27].

## Conclusions

An optimal controller was developed for the SSF process. The control problem was formulated as a scalar performance criteria minimization and was solved using an iterative algorithm based on steepest descent technique. Control profiles converged after 15 iterations. Simulations showed reduced glucoamylase requirement with optimum controller. A fermentation system was built and calibrated. Set point controllers for temperature and pH were tested. The temperature controller maintained temperature within ±0.5°C, while pH controller had a maximum deviation of ±0.2 from the setpoint. Use of optimal control algorithm for the SSF process resulted in lower peak glucose concentration, similar ethanol yields (13.38±0.36% v/v and 13.50±0.15% v/v for optimally controlled and baseline experiments respectively) and 50% reduction in glucoamylase amount required for SSF process under varying operating conditions as compared to standard SSF process.

Optimal controller significantly improved final ethanol concentrations as compared to conventional process without optimal controller under conditions of temperature and pH disturbances. Ethanol concentrations were lower for $E1T3_{OC,\,T}$ (13.35±1.28% v/v) as compared to $E1T1_{Baseline}$ (13.50±0.15% v/v). However final ethanol concentrations were significantly higher compared to $E2T1_{T,\,adjusted}$ (12.52±1.19% v/v) and $E2T2_{T,\,not\,adjusted}$ (12.21±0.22% v/v). Ethanol concentrations were lower for $E1T4_{OC,\,pH}$ (12.65±0.74% v/v) compared to $E1T1_{Baseline}$ (13.50±0.15% v/v), but was higher compared to $E2T3_{pH,\,adjusted}$(11.86±0.49% v/v) and $E2T4_{pH,\,not\,adjusted}$(12.44±0.33% v/v).

Use of the optimal controller in conventional dry grind ethanol process could result in lower glucoamylase dose, higher operating temperature and increased ability to minimize the impact of process disturbances. Measurable savings in lower enzyme usage and reduced cooling requirement could result in estimated cost savings up to \$1 million for a 151 million L/yr (40 million gal/yr) dry grind plant.

## Appendix A: Model Summary

The cybernetic yeast model equations are summarized below.

Cybernetic Control Equations

$$r_1 = \mu_1 e_1 \left(\frac{G}{K_1 + G}\right)\left(1 - \mathrm{H}_{G,150}\left(\frac{G-150}{650-150}\right)\right)\left(1 - \mathrm{H}_{E,95}\left(\frac{E-95}{150-95}\right)\right)$$

$$r_2 = \mu_2 e_2 \left(\frac{G}{K_2 + G}\right)\left(1 - \mathrm{H}_{G,150}\left(\frac{G-150}{650-150}\right)\right)\left(1 - \mathrm{H}_{E,95}\left(\frac{E-95}{150-95}\right)\right)$$

$$r_3 = \mu_3 e_3 \left(\frac{EM}{K_3 + EM}\right)\left(\frac{SM}{K_4 + SM}\right)$$

$$r_4 = \mu_4 e_4 \left(\frac{G}{K_1 + G}\right)\left(\frac{O}{K_5 + O}\right)$$

$$\mathrm{H}_{a,b} = \begin{cases} 1 \text{ if } a > b & \text{is the Heavyside function.} \\ 0 \text{ if } a \le b \end{cases}$$

$$v_1' = \left(\frac{r_1}{\mathrm{Max}\,(r_1, r_4)}\right) \quad \leftarrow \text{for substitutable pathway } r_1, r_4$$

$$v_1'' = \left(\frac{r_1/EM}{\mathrm{Max}\,(r_1/EM, r_2/SM)}\right) \quad \leftarrow \text{for complementary pathway } r_1, r_2$$

$$v_1 = v_1' v_1'' \quad \leftarrow \text{overall regulation for substitutable and complementary pathways } r_1$$

$$v_2 = \left(\frac{r_2/SM}{\mathrm{Max}\,(r_1/EM, r_2/SM)}\right) \quad \leftarrow \text{for complementary pathway } r_2, r_1$$

$$v_3 = 1 \quad \leftarrow \text{for pathway } r_3$$

$$v_4 = \left(\frac{r_4}{\mathrm{Max}(r_1, r_4)}\right) \quad \leftarrow \text{for substitutable pathway } r_4, r_1$$

$$u_1' = \left(\frac{r_1}{r_1 + r_4}\right) \quad \leftarrow \text{for substitutable pathway } r_1, r_4$$

$$u_1'' = \left(\frac{r_1/EM}{r_1/EM + r_2/SM}\right) \quad \leftarrow \text{for complementary pathway } r_1, r_2$$

$$u_1 = u_1' u_1'' \quad \leftarrow \text{overall regulation for both pathways } r_1$$

$$u_2 = \left(\frac{r_2/SM}{r_1/EM + r_2/SM}\right) \quad \leftarrow \text{for complementary pathway } r_2, r_1$$

$$u_3 = 1 \quad \leftarrow \text{for pathway } r_3$$

$$u_4 = \left(\frac{r_4}{r_1 + r_4}\right) \quad \leftarrow \text{for substitutable pathway } r_4, r_1 \tag{9}$$

Dynamic Mass Balance Equations

$$\text{Cell mass:}\quad \frac{\mathrm{d}X}{\mathrm{d}t} = (r_3 - D)\,X + 0.1212\,\eta_3\left(\frac{T-15}{30-15}\right) - \eta_4\left(\mathrm{H}_{T,33}e^{\left(\frac{T-33}{41-33}\right)}\right) - \mathrm{H}_{C_{aa},5}\mathrm{H}_{4.71,pH}0.05$$

$$\text{Glucose:}\quad \frac{\mathrm{d}G}{\mathrm{d}t} = \mathrm{H}_{GP,0}\,\frac{\mathrm{d}GP}{\mathrm{d}t} - \left(\frac{r_1\,\nu_1}{Y_1} + \frac{r_2\,\nu_2}{Y_2} + \frac{r_4\,\nu_4}{Y_3}\right)X + (G_0 - G)\,D$$

$$\text{Ethanol:}\quad \frac{\mathrm{d}E}{\mathrm{d}t} = \left(\phi_1\,\frac{r_1\,\nu_1}{Y_1}\right)X - E.D$$

$$\text{Oxygen}\quad \frac{\mathrm{d}O}{\mathrm{d}t} = -\left(\phi_2\,\frac{r_4\,\nu_4}{Y_3}\right)X + \left(K_{La}O^* - D.O\right)$$

$$\text{Energy precursors:}\quad \frac{\mathrm{d}EM}{\mathrm{d}t} = \left(r_1\,\nu_1 + r_4\,\nu_4 - \frac{r_3\,\nu_1}{\alpha_1}\right)X - \eta_1 \times \left(\frac{1229.56\,C_{aa}}{10^{pH-4.71}+1}\right) - EM.D$$

$$\text{Structural precursors:}\quad \frac{\mathrm{d}SM}{\mathrm{d}t} = \left(r_2\,\nu_2 - \frac{r_3\,\nu_1}{\alpha_2}\right)X - \eta_2\mathrm{H}_{pH,5.0}(0.725pH) - SM.D$$

$$\text{Enzyme 1 for pathway } r_1\text{:}\quad \frac{\mathrm{d}e_1}{\mathrm{d}t} = u_1\left(\frac{G}{K_1+G}\right) - e_1\,\beta + \alpha^* - e_1.D$$

$$\text{Enzyme 2 for pathway } r_2\text{:}\quad \frac{\mathrm{d}e_2}{\mathrm{d}t} = u_2\left(\frac{G}{K_2+G}\right) - e_2\,\beta + \alpha^* - e_2.D$$

$$\text{Enzyme 3 for pathway } r_3\text{:}\quad \frac{\mathrm{d}e_3}{\mathrm{d}t} = u_3\left(\frac{EM}{K_3+EM}\right)\left(\frac{SM}{K_4+SM}\right) - e_3\,\beta + \alpha^* - e_3.D$$

$$\text{Enzyme 4 for pathway } r_4\text{:}\quad \frac{\mathrm{d}e_4}{\mathrm{d}t} = u_4\left(\frac{G}{K_1+G}\right)\left(\frac{O}{K_5+O}\right) - e_4\,\beta + \alpha^* - e_4.D$$

$$\text{Acetic acid:}\quad \frac{\mathrm{d}C_{aa}}{\mathrm{d}t} = -D.C_{aa} + \left(\mathrm{H}_{5.0,pH}(0.1056) + \mathrm{H}_{pH,5.0}\,(0.0533pH - 0.1782)\right)\frac{\mathrm{d}X}{\mathrm{d}t}$$

$$\text{Glycerol:}\quad \frac{\mathrm{d}C_{gy}}{\mathrm{d}t} = -D.C_{gy} + \left(\mathrm{H}_{5.0,pH}(4.018) + \mathrm{H}_{pH,5.0}\,(0.416pH - 1.40)\right)\frac{\mathrm{d}X}{\mathrm{d}t}$$

$$\text{Saccharification:}\quad \frac{\mathrm{d}GP}{\mathrm{d}t} = (GP_0 - GP)\,D + \left(GA_{Activity}GA\right) \tag{10}$$

**Table 4** Parameters used for the SSF simulations

| Parameter values |
|---|
| $\alpha^*$ (g/L.s) = $7.04619\times10^{-3}$ |
| $\beta$ (1/s) = 0.095796 |
| $D$ (1/s) = 0.0 |
| $K_{La}$ (1/s) = $3.5\times10^{2}$ (aerobic fermentation) |
| $K_{La}$ (1/s) = 0.0 (anaerobic fermentation) |
| $K_1$ (g/L) = 71.0798 |
| $K_2$ (g/L) = 99.7452 |
| $K_3$ (g/L) = 10.96 |
| $K_4$ (g/L) = 8.14321 |
| $K_5$ (g/L) = $1.08898\times10^{-3}$ |
| $Y_1 = 5.00602\times10^{-2}$ |
| $Y_2 = 1.68912\times10^{-1}$ |
| $Y_3 = 1.07946\times10^{-1}$ |
| $\phi_1 = 5.15\times10^{-1}$ |
| $\phi_2 = 5.50923\times10^{-1}$ |
| $\mu_1$ (1/s) = 0.268862 |
| $\mu_2$ (1/s) = 0.226583 |
| $\mu_3$ (1/s) = 0.363602 |
| $\mu_4$ (1/s) = 0.355017 |
| $\alpha_1 = 1.33103$ |
| $\alpha_2 = 1.09596$ |
| $\eta_1$ (1/s) = $3.3349\times10^{-3}$ |
| $\eta_2$ (g/L·s) = $1.2137\times10^{-3}$ |
| $\eta_3$ (g/L·s.°C) = $1.0862\times10^{-2}$ |
| $\eta_4$ (g/L·s) = $9.75287\times10^{-4}$ |
| $\eta_4$ (g/L·s) = $5.0\times10^{-4}$ |
| $V_{max}$ (g/L-s) = $2.34245\times10^{-2}$ |
| $K_{gp}$ (g/L) = 29.2525 |
| $K_g$ (g/L) = 13.9939 |

## List of Symbols

| | |
|---|---|
| $e_i$ | Concentration of $i$th enzyme (g/g cell mass) catalyzing reaction $r_i$ |
| $r_i$ | Reaction rate for $i$th pathway (1/s) |
| $t$ | Time (s) |
| $C_{aa}$ | Concentration of acetic acid (g/L) |
| $C_{gy}$ | Concentration of glycerol (g/L) |
| $D$ | Dilution rate (1/s) |
| $E$ | Concentration of ethanol (g/L) |
| $EM$ | Concentration of energy metabolite precursors (g/L) |
| $G$ | Concentration of glucose (g/L) |
| $GA$ | Concentration of glucoamylase (g/L) |
| $GP$ | Concentration of glucose equivalent of dextrins(g/L) |
| H | The Heavyside function |
| $K_i$ | Monod model constants (g/L) |
| $K_{gp}$ | Monod model constant in saccharification model(g/L) |
| $K_g$ | Product inhibition constant in saccharification model(g/L) |

| | |
|---|---|
| $K_{La}$ | Oxygen mass transfer coefficient (1/s) |
| $O$ | Concentration of oxygen (g/L) |
| $O^*$ | Dissolved oxygen concentration limit (g/L) |
| $P_{ij}$ | Concentration for product produced in *i*th pathway and *j*th reaction |
| $pH$ | Mash/fermenter pH |
| $SM$ | Concentration of structural metabolite precursors (g/L) |
| $T$ | Mash/fermenter temperature (°C) |
| $V_{max}$ | Rate constant in saccharification model(g/L-s) |
| $X$ | Concentration of cell mass (g/L) |
| $Y_i$ | Yield coefficient for *i*th pathway |
| $\alpha_1,\ \alpha_2$ | Coefficients for production of a unit cell mass from EM and SM in reaction $r_3$, respectively |
| $\alpha^*$ | Constant intracellular enzyme synthesis rate (g/L·s) |
| $\beta$ | Constant intracellular enzyme breakdown rate (1/s) |
| $\eta_1$ (1/s), $\eta_2$ (g/L·s), $\eta_3$ (g/L·s·°C), $\eta_4$ (g/L·s) | Assumed proportionality constants for including the environmental effects |
| $\mu_i$ | Growth rate constant for *i*th pathway (1/s) |
| $\nu$ | Cybernetic variable controlling enzyme activity |
| $u$ | Cybernetic variable controlling enzyme synthesis |
| $\phi_1$ | Coefficients for production of ethanol in reaction $r_1$ |
| $\phi_2$ | Coefficients for consumption of oxygen in reaction $r_4$ |
| *Superscripts* | |
| c | Complementary pathway |
| s | Substitutable pathway |
| n | Number of reactions/alternative pathways |

## References


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