Modeling and Predictive Control of a Rotating Disk Bioreactor

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Abstract—**A rotating disk bioreactor (RDB) produces cohesive cellulose gels rapidly because of the high surface area, high volumetric efficiency, and low power consumption. A novel feature of the RDB that we have developed is that solids added to the medium enter the gel and are held at selected locations. Different solid materials such as silica gel, glass spheres, metallic powders, carbon, and common plant cellulose can be incorporated into the gel and gradients, stripes or bands of solids can be formed, resulting in a new type of biomaterial with applications in foods, medicine, bioprocessing, and manufacture of novel forms of paper. Incorporation of solid particles into the gelatinous matrix of bacterial cellulose involves complicated fluid/particle hydrodynamics. Experimental results are shown for a semibatch RDB, while simulation studies apply model predictive control (MPC) to a continuous RDB. Two MPC approaches are developed and analyzed for setpoint tracking and disturbance rejection.**

I. Introduction

A. Background Information

Bacterial cellulose is used in foods, acoustic diaphragms for audio speakers or headphones, high-strength specialty paper, and has medical applications such as wound dressings and artificial skin (Brown, 1992; Okiyama et al., 1993; Takai, 1994; Yamanaka and Wantanabe, 1994). Most of the bacterial cellulose that is currently an article of commerce comes from agitated, deep-culture fermentation with strains of *Acetobacter xylinum* that form no film but produce reticulated fibers. Pellets made in an air-lift fermentor have properties that fall in between the gelatinous mat from surface cultures and the fibrous version (Chao et al., 2000).

In static cultures, *A. xylinum* produces cellulose that extracellularly assembles into a thick mat, known as a *pellicle*. Yamanaka et al. (1989) observed that bacterial cellulose displays a Young's modulus value (a measure of shape retention) of 30 GPa, approximately 4 times greater than any organic fiber. The measured tensile strength of the treated material is about five times greater than polyethylene or vinyl chloride films (Japan Industrial Journal May 15, 1987).

B. Motivation and Significance of the Process

Production of a totally new class of composite biopolymers with a simple, low-cost device should have theoretical interest and unlimited commercial potential. We have a unique method for making composites of strong, tough bacterial cellulose with a wide range of particles, both organic and inorganic. The particles within the cellulosic matrix can be in stripes, gradients, mixtures of different sizes, mixtures of different particles, and in controlled concentrations. The organisms grow in a slime that attaches tenaciously to a support, their voracious need for oxygen encourages polymer production, potential contaminating organisms are denied good access to oxygen, and the alternative of a stirred tank reactor is unacceptable because it cannot supply oxygen without undesirable agitation that impairs the formation of a gel or pellicle.

The rotating disk bioreactor has unique advantages when the product attaches to the disks because of the association of production and aeration. Our results with cellulose already surpass the alternative of production by surface cultures in a tray reactor. Feeding, bleed of spent medium, product harvesting, and process control impact on new applications and present engineering challenges.

Of the many potential applications of this technology, the most appealing are:

- particles to which enzymes are immobilized to carry out sequential bioreactions,
- encoding of paper with a particle signature to combat counterfeiting,
- manufacture of elegant grades of paper for which scrap newsprint is fully acceptable as a filler,
- paper with metallic particles for controlled dielectric constant and conductivity for electronic applications,
- abrasive papers with the particles inside instead of simply glued to the surface,
- medical coverings such as wound dressings or artificial skin with time-release particles,
- gels of controlled permeability and/or reactivity of embedded particles for downstream processing.

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II. Rotating Disk Bioreactor

Our group has developed a rotating disk bioreactor with perforated disks that allow film growth on both sides. Disks (as few as one or as many as a dozen) are on a shaft that turns so that they dip into the nutrient medium, continue up into the air, and dip again, as shown in Figure 1. Bacteria attach to the disks and begin to form cellulose as a gel that increases in thickness. Solid disks are inferior to disks that are perforated to allow the gel to grow through for strong attachment. Rotational speed for optimal film production is about 12 rpm, but intact films have been produced at rates of up to 24 rpm.

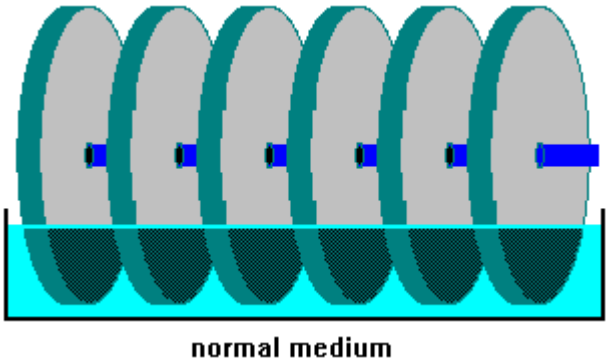

Figure 1. Rotating disk bioreactor.

A schematic of the automated bioreactor is shown in Figure 2 (Serafica et al., 2002). The measured variables include temperature, pH, turbidity and oxygen concentration, and the manipulated inputs include feed flowrate, caustic addition rate, particle addition rate, and heat flux (from a heating tape).

A premier feature of the design is the capability of changing the liquid medium conditions during production to modify the cellulose gel to incorporate particles at selected locations in the gel, for forming bands or stripes of solids, as shown in Figure 3.

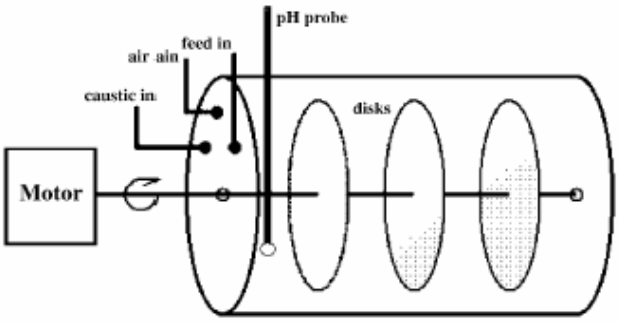

Figure 2. Automated rotating disk bioreactor.

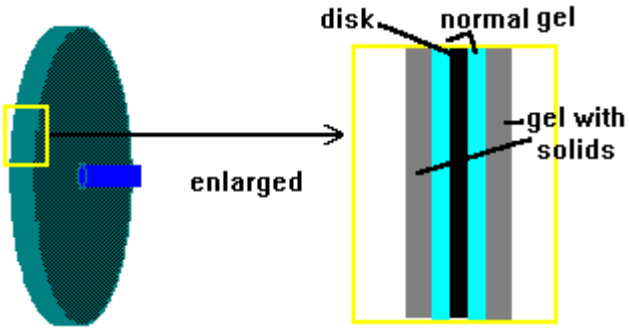

Figure 3. Depiction of growing gel, with bands of particles.

III. Experimental Methods

Oxygen tension in the medium (mg/l) was measured with YSI oxygen meter Model 54A. Typical ranges of 1-5 mg/liter were observed. The conclusion is that oxygen is not rate-limiting for cellulose formation. Glucose was analyzed with a YSI Model 2700 Glucose Analyzer. All samples were either filtered or centrifuged prior to injection and diluted to the appropriate range of 0.1 to 40 g/l glucose.

The cellulose pellicle is measured at the end of each run. The cellulose is normally rinsed with water to remove any residual sugar and boiled in 2% NaOH solution for 1 hr to release cells from the cellulose fibrils. Washing with deionized water removes the residual base. Wet weight is measured after draining for five minutes. The dry weight is taken after drying overnight at 95C.

Rapid cellulose production occurs between pH of 3.5 to 7 with the highest rate of formation at around pH 4.5 to 6. These data agree with previous investigations (Masaoka et al., 1993), and there seems to be a consensus regarding the optimum pH range for cellulose production (Embuscado et al., 1994, Lapuz et al., 1967). In static cultures, the cells produced a thicker cellulose pellicle when grown in fructose than in glucose, and sucrose produced the thinnest film. However, based on dried pellicles, all three sugars exhibited similar cellulose production rates. The final pH was lowest for glucose because of gluconic acid production. Observations using atomic force microscopy (AFM) showed the film product by the rotating disk bioreactor to be more open with less assembled fibrils than those synthesized in static cultures. The cell density in the film is also noticeably lower in the rotated film than in the static system. The fibrils are also more organized in the static films than in rotating disk system.

The most important characteristic of this bioreactor design is capability of changing the medium condition and composition without disrupting the pellicle growth. Various materials were added directly to a growing film. Materials with molecular weights ranging from 50,000 to 100,000 such as dyes, proteins, dextran, polyethylene glycol and carboxymethyl cellulose diffused freely through the gel. Yeast and bacteria also moved freely. India ink showed some trapping, but some particles diffused.

Activated carbon (50-100 mm) and glass beads (120-250 mm) were trapped.

An example closed-loop response is shown in Figure 4, where the concentration of particles in solution (based on turbidity as measured by a Milton-Roy Spectronic 21 spectrophotometer) is controlled by manipulating the particle addition rate. The concentration of particles in the growing cellulose gel is directly related to the concentration of particles in solution.

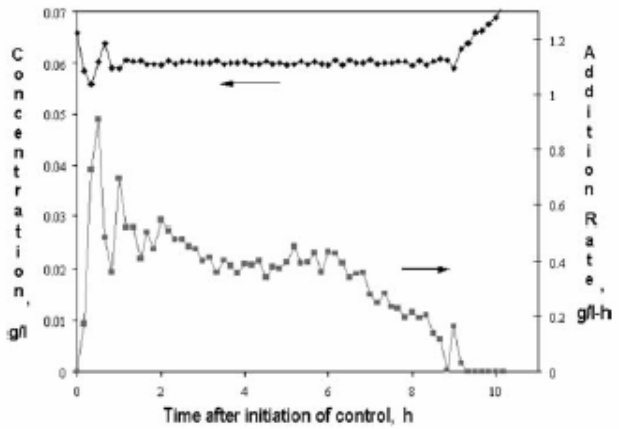

Figure 4. Closed-loop control of solution particle concentration, using a proportional-integral controller.

Recent experiments have measured rates of incorporation of plant cellulose into microbial cellulose, and there exists an optimum rotational speed of the disks to maximize the uptake rate (Mormino and Bungay, 2003).

IV. Mathematical Modeling

Our preliminary mathematical model of growth focused on the kinetics of cellulose production in individual Acetobacter cells based on mass balances for the overall system and around the particle. The model was extended to cover the different stages of production of the bacterial cellulose film/pellicle in static culture. The current rotating disk model includes important parameters such as rotational speed for calculating cellulose production rates, disk area for cellulose production, and kinetic growth rate of cellulose (Mormino, 2002).

In the simulation results that follow, we assume that the sugar concentration is high enough that the growing cellulose gel is on the "zero order" portion of the growth curve, that is, the gel growth rate is constant.

Overall and solid particle balances are shown in equations 1 and 2. The last term in (2) accounts for the uptake of particles in the growing cellulose gel.

$$\frac{dV}{dt} = F_H + F_D - F_O \tag{1}$$

$$\frac{dx_p}{dt} = \frac{1}{V}\left(F_H x_{pf} - F_H x_p - F_D x_p - k_p A_d x_p\right) \tag{2}$$

Where V is the liquid volume, x_p is the particle concentration in solution, k_p is a particle uptake rate constant, A_d is the total disk area, and F_H, F_D and F_O are the volumetric flowrates of the concentrated particle feed, dilute sugar feed and outlet streams, respectively. The parameter and steady state values used in this study can be found in Table 1.

Table 1: Parameters and Steady State Values

V_s	1 liter
x_{Ps}	2 grams/liter
x_{Pf}	20 grams/liter
A_d	364.83 cm^2
k_p	1.33e-06 liters/min/cm^2
F_{Hs}	0.0034 liters/min (V/F = 30 min)
F_{Ds}	0.03 liters/min (V/F = 30 min)
F_{Os}	0.0334 liters/min (V/F = 30 min)
F_{Hs}	4.6519e-04 liters/min (V/F = 4 hrs)
F_{Ds}	0.0037 liters/min (V/F = 4 hrs)
F_{Os}	0.0042 liters/min (V/F = 4 hrs)

The preliminary simulation results that follow are based on equations 1 and 2. In order to control properties related to the growing cellulose film, more detailed modeling equations are needed. A major assumption is that growing cells (biomass) have already attached themselves to the rotating disk, and that the "lag phase" has been completed; that is, the reactor has achieved steady-state conditions for cell growth. A balance on the substrate, glucose, yields

$$\frac{dx_g}{dt} = \frac{1}{V}\left[F_D x_{gf} - F_D x_g - F_H x_g - \frac{\mu_{\max}\overline{m}A_d}{Y_{bm/g}}\right] \tag{3}$$

where μ_{max} is the specific growth rate, $\overline{m}$ is the mass of active biomass per unit disk area (assumed to be constant at a quasi-steady state value), and $Y_{bm/g}$ is the yield of biomass from glucose. Studies by Mormino (2002) indicate that this glucose conversion term is 0.4 g/hr over a wide range of glucose concentrations.

The mass of dry cellulose, and the particle mass, in the growing gel are found from the following equations

$$\frac{dM_c}{dt} = \frac{\mu_{\max}\overline{m}A_d}{Y_{c/g}} \tag{4}$$

$$\frac{dM_p}{dt} = k_p A_d x_p \tag{5}$$

and the ratio of particles to cellulose at any point in the gel is $y_p = M_p/M_c$. Previous studies have shown that the production of cellulose is primarily by the cells in the growing gel, and not in the solution.

Gluconic acid is a by-product of the glucose conversion; here we assume that pH is tightly regulated by

manipulating the flowrate of a caustic feedstream. These equations are neglected in our current model.

V. CONTROL

A. Model Predictive Control

Control of the rotating disk bioreactor was performed by using a model predictive control (MPC) approach. In model predictive control, the process outputs are predicted a specified number of steps into the future using a representative process model. The objective is to minimize the sum of squared deviations from a specified reference trajectory. The objective function is defined as:

$$J = \sum_{i=1}^{P} (R - \hat{y})^T W_y (R - \hat{y}) + \sum_{i=1}^{M} \Delta u^T W_u \Delta u \qquad (6)$$

Where P is the prediction horizon, M is the control horizon, Δu is the vector of optimal control moves, and W_y and W_u are output and input weighting matrices respectively. A more complete description of model predictive control has been given by Morari and Lee (1999) and Muske and Rawlings (1993).

There are a variety of models that can be chosen for use in the model predictive control algorithm. One of the most common is a state space model with an additive disturbance term, similar to the assumption in dynamic matrix control (DMC, Cutler and Ramaker, 1980). The state space DMC model is defined as:

$$\begin{aligned} x_{k+1} &= \Phi x_k + \Gamma u_k \\ d_{k+1} &= d_k \\ y_{k+1} &= C x_{k+1} + D u_{k+1} + d_k \end{aligned} \qquad (7)$$

One disadvantage to using DMC is that the disturbance term only has the ability to update the predicted outputs. The states remain "open-loop" in this formulation. This will have an impact on the types of disturbances that DMC is able to reject, and the ability to control unmeasured variables. In order to achieve better disturbance rejection, a state space model augmented with a Kalman filter can be used (Muske and Badgwell, 2002). In this case, the Kalman filter will be used to estimate a disturbance state. The augmented state space system is, where w_k and v_k are zero-mean white noise terms with covariances Q and R respectively:

$$\begin{aligned} \begin{bmatrix} x_{k+1} \\ d_{k+1} \end{bmatrix} &= \begin{bmatrix} \Phi & \Gamma_d \\ 0 & I \end{bmatrix} \begin{bmatrix} x_k \\ d_k \end{bmatrix} + \begin{bmatrix} \Gamma \\ 0 \end{bmatrix} u_k + \begin{bmatrix} 0 \\ 1 \end{bmatrix} w_k \\ y_k &= \begin{bmatrix} C & 0 \end{bmatrix} \begin{bmatrix} x_k \\ d_k \end{bmatrix} + v_k \end{aligned} \qquad (8)$$

Using augmented state notation:

$$x_k^a = \begin{bmatrix} x_k \\ d_k \end{bmatrix}, \Phi^a = \begin{bmatrix} \Phi & \Gamma_d \\ 0 & I \end{bmatrix}, \Gamma^a = \begin{bmatrix} \Gamma \\ 0 \end{bmatrix}, C^a = \begin{bmatrix} C & 0 \end{bmatrix} \qquad (9)$$

The augmented states are updated using the predictor/corrector equations:

$$\begin{aligned} \hat{x}_{k|k-1}^a &= \Phi^a \hat{x}_{k-1|k-1}^a + \Gamma^a u_{k-1} \\ \hat{x}_{k|k}^a &= \hat{x}_{k|k-1}^a + L_k \left(y_k - C^a \hat{x}_{k|k-1}^a \right) \end{aligned} \qquad (10)$$

The L_k term is a Kalman gain, which can be found recursively at each time step, or can be assumed constant by solving the steady-state Riccati equation (as is done in this work). In the simulations that follow, we use the linear model for the MPC computations, but use the original nonlinear equations to represent the plant.

B. Setpoint Tracking

The first test of the control algorithm is its ability to track output setpoints. This is of key importance in the rotating disk bioreactor because of the time-varying gel concentration profile that will be required during each run. Using the state space model with augmented states, a series of particle concentration setpoint changes were made. The resulting tracking of these setpoints, with corresponding required manipulated input profiles are shown in Figure 5.

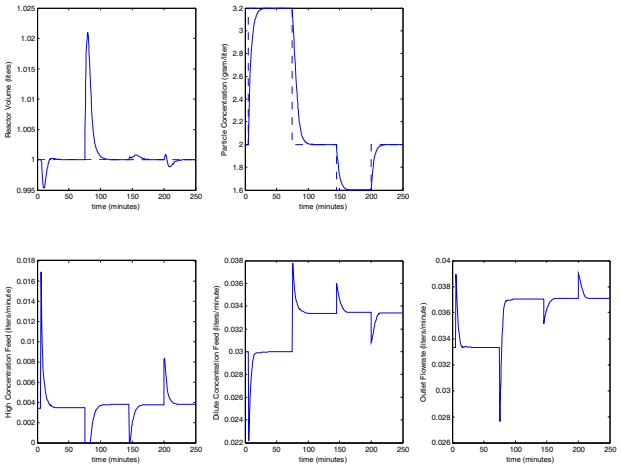

Figure 5. Setpoint tracking of particle concentration.

C. Continuous vs. Semi-batch Operation

In the previous results of Mormino, the rotating disk bioreactor was run in semi-batch mode. The particle concentration was controlled by continuous feeding an inlet stream, and there were no outlet streams. This leads to an inherent disadvantage of semi-batch operation. With no outlet stream, the volume of the bioreactor is not controlled and will continue to increase until it reaches the maximum bioreactor volume. In the aforementioned

continuous formulation, volume is included as a second controlled output, hence there are no volume limitations. This is illustrated in Figure 6.

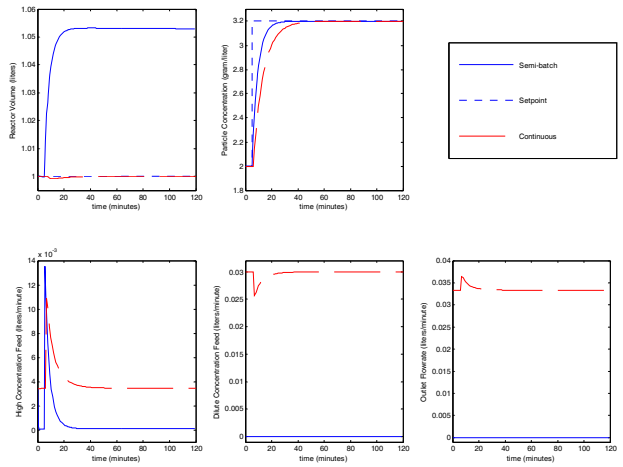

Figure 6. Continuous and semi-batch operation.

The advantage to continuous operation can clearly be seen in the figure. While both modes of operation are able to track particle concentration setpoints, semi-batch operation does so at the cost of requiring a volume that has already increased by 6% of the nominal volume for only one step change.

D. Input Disturbance Rejection

The second class of disturbance that it will be important to reject in the rotating disk bioreactor is input disturbances. These are disturbances that directly affect the inputs to the system. In the rotating disk bioreactor, these disturbances can take the form of an inlet flow rate disturbance or a unequal concentration in the high concentration feed stream F_H due to non-uniform mixing in the feed reservoir. Both DMC and augmented state Kalman filter models were simulated for a 10% disturbance in the high concentration feed stream flowrate, F_H. The simulations can be seen in Figure 7.

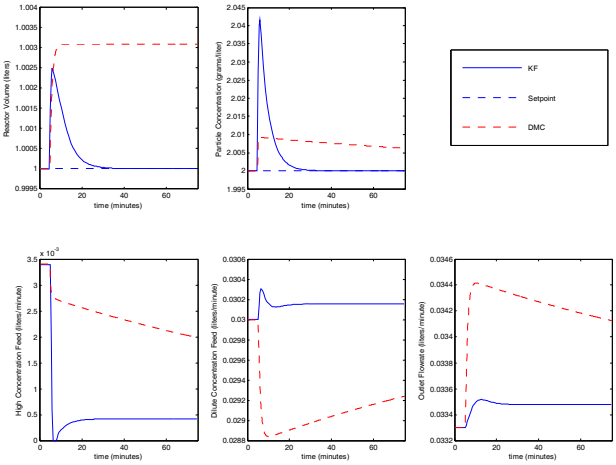

Figure 7. Comparison of input disturbance rejection.

While both DMC and augmented state space with Kalman filtering are able to reject the disturbance effects to the particle concentration, only the augmented state space with Kalman filtering is able to successfully reject the disturbance effects in volume as well. This is due to the Kalman filter updating the states of the system, which is the main advantage to the Kalman filtering approach referenced earlier. To be fair, it should be noted that the DMC approach can easily be extended to integrating systems.

E. Kalman Filter Tuning

In addition to being designed to handle both input and output disturbances, another benefit to using the augmented state space with Kalman filtering approach is that there is a built-in tuning mechanism based on process knowledge. The relative weights on the Q and R covariance matrices can be tuned depending on the measurement noise known to be present in the system. In MIMO systems like the rotating disk bioreactor, Q and R are both diagonal matrices with non-zero weighting. The dimension of Q is equal to the number of disturbances being estimated, and the dimension of R is equal to the number of measured outputs. Muske and Badgwell (2002) have shown that the number of estimated disturbances cannot exceed the number of measured outputs. A large Q weighting relative to R indicates that the measured outputs are to be trusted more than model outputs, which means very little measurement noise. A small Q weighting relative to R indicates that the model outputs are to be trusted more than measured outputs, which means a large degree of measurement noise. This principle is demonstrated on the rotating disk bioreactor in Figure 8.

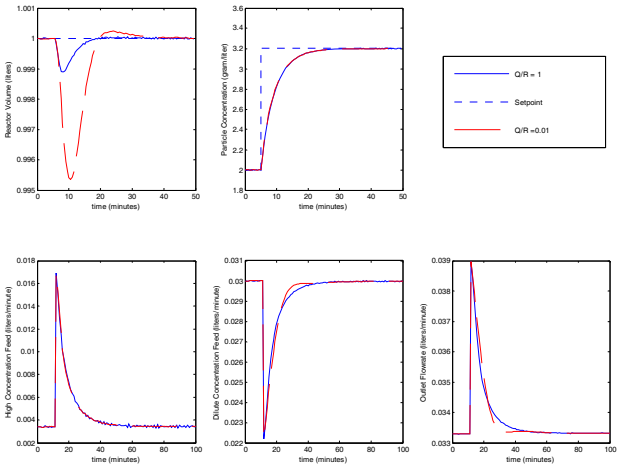

Figure 8. Comparison of Kalman tuning weights.

Figure 8 clearly shows that better setpoint tracking for a higher Q weighting, which is expected because the system had measurement noise of 1% of the steady state values.

F. Residence Time Effects

One of the parameters that plays a crucial role in the dynamics of the system is the ratio of reactor volume to flowrate, or the residence time. In the previous simulation results presented, a residence time of 30 minutes was used. There are benefits to analyzing the effect of residence time on control, as residence time can affect, among other things, the conversion of glucose. Figure 9 highlights the differences in dynamic responses to varying values of residence time.

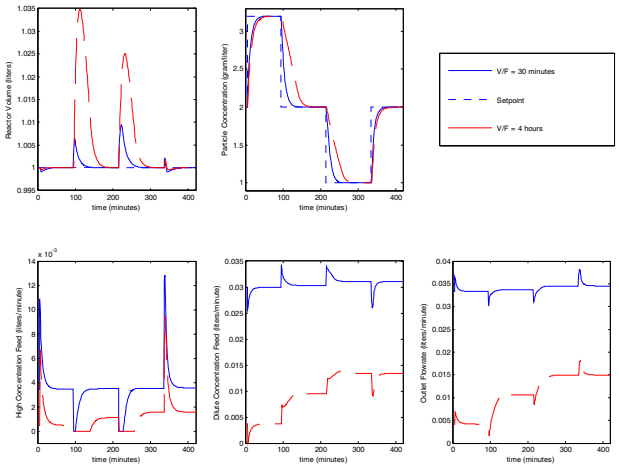

Figure 9. Comparison of residence times.

As can be clearly seen in Figure 9, the increased residence time of 4 hours has a slower response to setpoint changes than does the residence time of 30 minutes used previously. This slower response will need to be traded off against gains from increased glucose conversion to find an optimal residence time for a given system.

VI. Summary and Current Work

Rotating disk bioreactors produce cohesive cellulose that can be used in the bioprocessing, medicine, food production and paper manufacturing. Current experimental procedures run the rotating disk bioreactors in semibatch mode.

The ability to transition from batch to continuous operation of rotating disk bioreactors offers the promise of better control over the gel particle concentration profile. A first principles mathematical model of the rotating disk bioreactor was derived. The model was then used in concert with two different model predictive control (MPC) formulations. The augmented state space with Kalman filtering was shown to have superior performance compared to DMC for typical expected operating conditions including setpoint tracking and disturbance rejection.

In current work we are studying the effect of residence time, and developing a more comprehensive model for cellulose growth and incorporation of particles into the growing gel.

VII. Acknowledgment

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