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Multi-scale Framework for Modeling and Control of Fermentation Processes

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Abstract: In this paper, we propose a generalized multi-scale modeling framework for a continuous alcoholic fermentation using *Saccharomyces cerevisiae*. Based on the developed multi-scale modeling framework, a multi-scale control (MSC) strategy using PID-type controllers is then designed and compared with that of a single-scale control (SCC) strategy. Results indicate that MSC strategy could greatly improve the closed-loop performance. Also, with the right choice of control strategy by embedding micro-scale controller, this study shows that more complex controller algorithms might not be necessary.

1. INTRODUCTION

Rapid progress in the fields of biotechnology such as biochemistry, molecular biology, and metabolic engineering has led to a tremendous growth of knowledge concerning many biological systems of interest. One particular example is the *cell factory* systems for the production of metabolites, e.g. ethanol, lysine and hosts of fine chemicals. Unfortunately, the realization of this improved knowledge at the process system engineering level with the aims to improve product yield and productivity has not yet matched this fast growing knowledge. One of the key obstacles towards the widespread applications of this multidisciplinary pool of information is the current difficulty of integrating this knowledge, which is often characterized by multiple time and length scales. These scales are ranging from nano-scale at the *omic* level, to micro-scale at the cellular level and further up to macro-scale resolution at the plantwide level (Zhang et al., 2006).

Today, with the increasing challenges to meet stringent product quality in ever competitive market environments, we also need for a more rigorous modeling framework that could help us to develop deeper understanding about the system of interest. Interestingly, recent advances in the multi-scale modeling have provided an avenue for unifying this multi-scale information within a coherent framework, which not only allows for rigorous analysis but also sets up a direction for further research and development (Pablo, 2005; Kitano, 2002). Recent report in Ingram et al. (2004) provided an excellent review on the progress and classifications of multi-scale modeling. Moreover, with respect to the complex bioprocessing systems, the combined modeling technique and statistical tools can complement purely empirical procedures, accelerate and simplify process development, and open access to a better process understanding (Brass et al., 1997).

In the last three decades, the macro-scale approach of Moser (Starzak et al., 1994) has been extensively used by the process system engineering communities to describe microbial growth in the fermentation processes largely due to its simplicity. This normally led to unstructured type of kinetics models where the parameters, i.e. specific growth and product formation rates, linking the microbial metabolism with the bioreactors (macro-scale) conditions such as pH, temperature and substrate concentration are identified. In this approach, the living cell is treated as *black box* i.e. the intermediate reactions steps occurring within the cell are ignored. In view of the complex biological system at the micro-scale level which is generally characterized by numerous enzymatic reactions, the validity of such macro-scale model as a tool for bioreactor optimization and control analysis is particularly poor outside the region of its identification. Recent studies indicated that many of the process improvements which could not even be perceived from the macro-scale point of view, could be realized through a detailed analysis using the so-called metabolically structured model (micro-scale approach), i.e. minimization of glycerol in ethanol fermentation (Bideaux et al, 2006), optimal feeding strategy of lysine fermentation (Takiguchi et al., 1997), and strategy to maximize pentose fermentation in *Z. mobilis* (Altintas et al., 2006).

The search for strategies to improve bioprocessing yield and productivity could be broadly classified into two categories; (1) strain improvements via genetic modifications in a fixed extracellular environments, and (2) optimization and control of extracellular environments for a given strain (Galazzo and Bailey, 1990). With respect to the former approach, the metabolically structured model has been recognised as a vital tool to metabolic engineering in order to successfully improve the cellular functions of microbial strains of interest (Hatzimanikatis et al., 1998). Meanwhile the second approach is frequently adopted by the process engineering, in which case very often macro-scale models with regards to the bioprocess of interest are used as a tool for analysis in the bioreactor design, optimization and control.

While for decades the advanced control methods have been effectively employed for industrial chemical processing, the adoptions of these control techniques in biotechnological processes are still relatively rare (Komives and Parker, 2003). The vast majority of control strategies development for bioreactors still relies on the macro-scale models, and as such

the complex interactions between the extracellular environments with the thousands of intracellular enzymes and metabolites are generally ignored. Credible studies have shown that these multi-scale interactions could have profound impacts on the biological systems (e.g. Satroutdinov et al., 1992; Verduyn et al., 1992; Washburne et al, 1996). Only recently that some attempts have been reported on the use of multi-scale models which capture these interactions to improve bioreactor control performances e.g. (Soni and Parker, 2004; Teixeira et al., 2007).

One of the key research gaps in the current approach of using multi-scale model is the lack of emphasis on the multi-scale control structure analysis. More often, the model is used to develop the advanced controller techniques such as that of nonlinear model-based controllers. The basic tenet of this approach is that the control performance of complex biological system could somehow be improved through the adoption of advanced controller algorithms. But very often, the applications of these controller algorithms are sill facing various obstacles due to, for example the unresolved issue of nonconvex optimization in nonlinear predictive control. In the case of macro-scale system, it has been recognised that control structure selection could have far greater impact on the closed-loop performances than the choice of controller algorithms (Morari et al., 1980; Hovd and Skogestad, 1993). As for the multi-scale system, our recent works based on the *idealized* multi-scale model does indicate that judicial choice of multi-scale control structure can significantly contribute the overall closed-loop performance (Nandong et al., 2007). Additionally, with the right choice of control structure the use of complicated controller algorithms could be avoided i.e. just simple PID-type controllers could perform satisfactorily well.

With respect to some of the research gaps in the current multi-scale modeling and applications, the objectives of this paper are two-folds. The first objective is to propose a generalized multi-scale framework for bioprocessing system. Secondly, using a multi-scale model of alcoholic fermentation process, a multi-scale control strategy is proposed. The performance of this control system would then be compared with the traditional macro-scale controllers. Additionally, some of the issues and opportunities pertaining to the multi-scale strategies would also be highlighted.

2. FRAMEWORK OF MULTI-SCALE MODELING

The overall goal of multi-scale modelling is to capture neither too much nor too little information about the processes. Hence, a trade-off between simplicity of the resulting model and the amount of embedded process information is required. Fig. 1 shows the generalized framework of multi-scale modeling for bioprocesses, starting from the macro-scale down to the micro-scale level.

In this proposed framework, the ultimate objective is to predict or analyze the bioreactor performances using multi-scale information. The performance indicators of bioreactor could be divided into two categories; (1) steady-state performance in terms of product yield, conversion and productivity, and (2) dynamic controllability i.e. how easy to control the system at desired conditions (Fararooy et al., 1993). In the chemical process industries, dynamic controllability is one of the key issues that determine plant profitability.

The mixing and process inputs are two major factors that determine bioreactors conditions and in turn affect the bioreactor performance. Subsequently, there are two ways to predict the impact of bioreactor conditions on bioreactor performance. The common practice is via the use of unstructured kinetics in conjunction with the bioreactor model i.e. macro-scale approach. For the second method, the detailed interactions between the bioreactor conditions and intracellular reactions are generally considered. In this case, at the meso-scale level, the effect of mixing and hence transport phenomena plays a very important role. Due to the deviation from ideal mixing behaviour, concentration and temperature gradients normally occur and lead to the formation of various zones inside the bioreactor. This effect is particularly important to industrial scale process where transport limitation is regarded as one of the major phenomena leading to process yield reduction (Vrábel et al., 2001). This number of zones depends on the degree of mixing where for the ideal case it would then reduce to a single zone. Consequently, each zone would have different physiological impacts upon the microbial activity. For example, in a zone where substrate is rich, an overflow metabolism (or *Crabtree* effect) can occur where in the case of *S. cerevisiae*, ethanol is produced even under aerobic conditions (Merico et al., 2007).

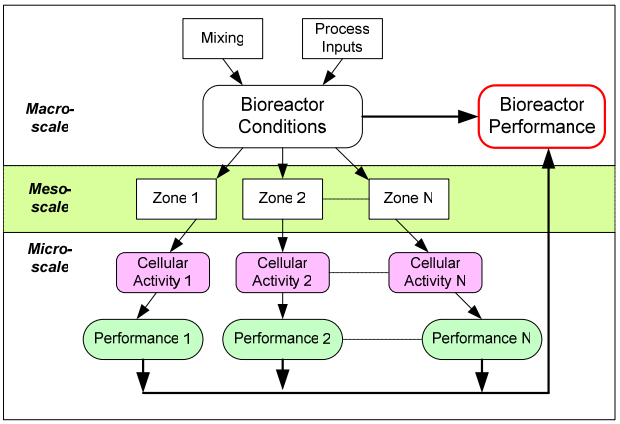

Fig. 1. Generalized framework of multi-scale modelling for bioprocessing system

Depending on the overall goal of the bioprocess, the cellular performances could be measured in terms of one or combination of factors such as growth rate, metabolite production rate, cell viability and selectivity for the case of multiple branched reactions. Finally, the multi-scale information could be integrated within a suitable model to predict or analyze the bioreactor performance at the macro-scale level.

2.1 Multi-scale Modelling of Continuous Alcoholic Fermentation - Example

The modelling of the continuous alcoholic fermentation assumes the following:

1. Homogeneous population and well mixing in bioreactor i.e. single zone in bioreactor.
2. For the cellular metabolism, only the reactions in the cytoplasm is considered i.e. focus on the ethanol fermentation under limited oxygen supply.
3. Concentrations of intracellular ATP, ADP, AMP and NADH are considered as micro-scale inputs to the model.
4. Fresh glucose concentrations (So), inlet flowrate of fresh feed (Fo) and outlet flowrate (F) are macro-scale inputs available for manipulation.
5. Reactions in cells follow the kinetics models of Rizzi et al (1996). Fig. 2 shows the simplified metabolic pathway reactions leading to the key product (ethanol) and by-product (glycerol). Interested readers could refer to Rizzi et al. (1997) for the detailed descriptions shown in Fig. 2.

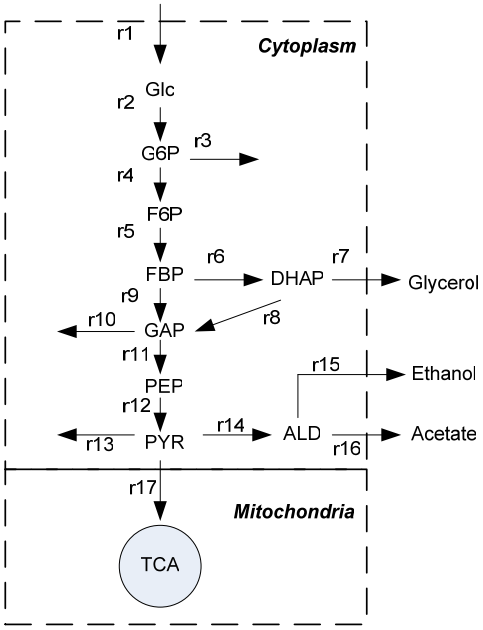

Fig. 2. Simplified metabolic pathways reactions of *S. cerevisiae*

The macro-scale mass balances for the extracellular product components which are ethanol (EtOH), glycerol (Gly) and acetate (Ace) and extracellular glucose (GlcE) are as reported by Rizzi et al (1996):

$$\frac{dEtOH}{dt} = r15\frac{C_x}{\rho} - \frac{F}{V}EtOH \tag{1}$$

$$\frac{dGly}{dt} = r7\frac{C_x}{\rho} - \frac{F}{V}Gly \tag{2}$$

$$\frac{dAce}{dt} = r16\frac{C_x}{\rho} - \frac{F}{V}Ace \tag{3}$$

$$\frac{dGlcE}{dt} = \frac{F}{V}(S_0 - GlcE) - r1\frac{C_x}{\rho} \tag{4}$$

The V, F, C_x, S_0 and ρ are the hold-up volume, outlet flowrate, cell concentration, inlet glucose concentration and cellular specific volume respectively.

Moreover, the biomass mass balance is expressed as:

$$\frac{dC_x}{dt} = \mu C_x - \frac{F}{V}\alpha C_x \tag{5}$$

Where the dynamic of bioreactor hold-up liquid level (L):

$$A\frac{dL}{dt} = F_o - F \tag{6}$$

Where A is cross-sectional area of the bioreactor and F could be expressed as a function of L and valve coefficient (k_v):

$$F = k_v\sqrt{L} \tag{7}$$

Where it is assumed that the specific growth rate is given by:

$$\mu = \frac{r3 + r13}{\rho}\left(1 - \frac{C_x}{C_{x\max}}\right) \tag{8}$$

The specific growth rate due other reaction pathways in the mitochondria is assumed negligible. The coefficient α in (5) is the cell washout factor i.e. the fraction of the biomass leaving the bioreactor. This normally leads to the high density of biomass in the bioreactor, which in turn tends to create less favourable conditions for growth. Hence, it is necessary to consider the inhibition effect of biomass on the specific growth rate. This biomass inhibition is assumed to take a linear form i.e. a ratio of C_x to the maximum achievable biomass concentration C_{xmax}.

As for the micro-scale mass balances of intracellular metabolites (c_i), 9 components (Glc, G6P, F6P, FBP, DHAP, GAP, PEP, PYR and ALD) in the cytoplasm are considered:

$$\frac{dc_i}{dt} = f(r_k) - \mu c_i \qquad i = 1, 2\ldots9 \text{ and } k = 1, 2\ldots17 \tag{9}$$

And the function $f(r_k)$ describes the reaction step r_k that involved in each metabolite production or consumption. The reaction rates (r_k) are obtained from Rizzi et al (1996). The macro-scale system or bioreactor is described by (1)-(6) and that of the micro-scale system is represented by (9). The specific growth rate (8) could be treated as the interface parameter linking micro- and macro-scale systems. The outputs of the micro-scale system are the reaction rates (r_k) which become the parameters to bioreactor model (1)-(6). In total there are 15 ODEs which represent the overall multi-scale system of fermentation process in this study. To avoid numerical computational problems, all the micro- and macro-scale ODEs are scaled appropriately such that, the order of intracellular metabolites is approximately similar to the order of extracellular output variables.

2.2 Multi-scale Input-Output Structure

Fig 3 displays the proposed multi-scale control structure in this case-study. There are three macro-scale outputs to be controlled; (1) ethanol concentration *EtOH* (2) extracellular glucose concentration *GlcE*, and (3) liquid level *L*. The manipulated-*controlled variables pairings are (1) Fo-EtOH,*

(2) So-GlcE, and (3) k_v-*L*. The valve coefficient (k_v) is used to adjust the outlet flowrate (*F*). The notation *R* is a vector of intracellular rates (r_k) which is considered output from the micro-scale point of view but is considered as parameters to macro-scale system. And the *X* is a vector of macro-scale conditions that have direct influence on the micro-scale system, e.g. extracellular glucose *GlcE*. To implement multi-scale control (MSC) strategy, the macro-scale Fo-EtOH controller is augmented with the micro-scale NADH-EtOH controller. The main assumption on the implementation of this strategy is that the co-factor NADH could be manipulated as input variable. So, for the single-scale control (SCC) strategy, the NADH-EtOH configuration is removed.

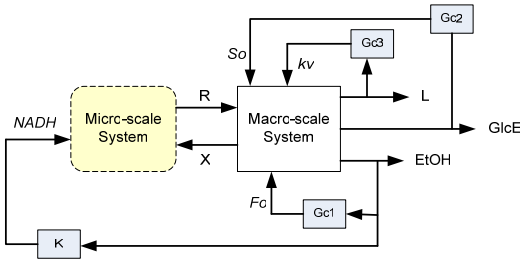

Fig. 3. Multi-scale input-output structure of fermentation considered in this case study

3. RESULTS

Table 1 shows the tuning values for the macro-scale controller configurations. In this case only PI-type controllers are employed. Note that, when the NADH-EtOH micro-scale controller is implemented for the case of MSC strategy, all of the tuning values of the macro-scale controllers are kept the same.

Table 1. Macro-scale controller tunings

Configuration	*Controllers*
Fo-EtOH	$G_{c1} = -0.015\left(5\times10^{3}s+1\right)/s$
So-GlcE	$G_{c2} = 0.094\left(-8.5s+1\right)/s$
kv-L	$G_{c3} = -0.095\left(9\times10^{2}s+1\right)/s$

As for the micro-scale NADH-EtOH configuration, P-only controller is employed for simplicity. No attempt is made in this case study to optimize the controller tuning values. The reason for this is that our key objective is to compare the closed-loop performance between SCC strategy and MSC strategy i.e. to investigate the potential benefit of the latter strategy based on the developed multi-scale model.

To assess the comparative performances between SCC strategy and MSC strategy, the control system are subject to two different output disturbances i.e. EtOH and GlcE output disturbances. Furthermore, these disturbances are assumed to enter the system as step inputs changes.

3.1 Closed-loop Performance under EtOH Output Disturbance

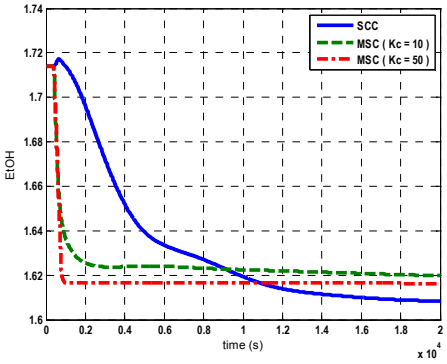

Fig. 4. Responses of scaled EtOH when subject to output disturbance of ΔEtOH = 0.1

Fig. 4 illustrates the comparative closed-loop performances on the scaled EtOH when the scaled output disturbance in EtOH is 0.1 at time of 400s. For the SCC strategy, it takes more than 20,000 seconds (about 6 hrs) for EtOH to settle. On the other hand, for MSC strategy with the micro-scale controller of Kc = 10, it only takes about 2,000 seconds (about 0.5 hrs) to settle. However, there is a minor offset. When the Kc for the micro-scale controller is increased to 50, the response of the closed-loop system is even faster with reduced offset i.e. settling time is less than 1,000 seconds.

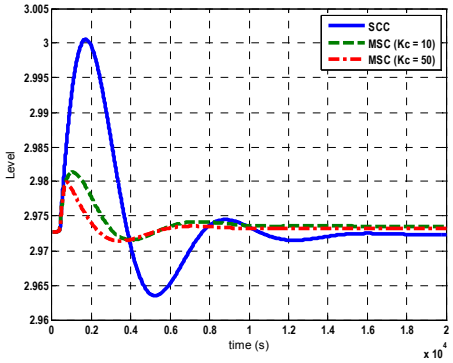

Fig. 5. Responses of scaled bioreactor liquid level when subject to output disturbance of ΔEtOH = 0.1

Fig. 5 shows the responses of the scaled L (liquid level) to the output disturbance in EtOH of 0.1. It can be noted that the MSC strategy leads to not only a faster response (shorter settling time) but also less oscillation with smaller overshoot. Fig. 6 indicates the responses of scaled GlcE when subjected to the disturbance in EtOH. Although there is no clear improvement on the closed-loop response of GlcE when the micro-scale controller is implemented, it is important to note that both SCC and MSC perform virtually the same. Also it is important to point out that the implementation of MSC remains stable even for the large change in Kc values and yet with the added advantages on the clear improvements on the EtOH and L control-loops.

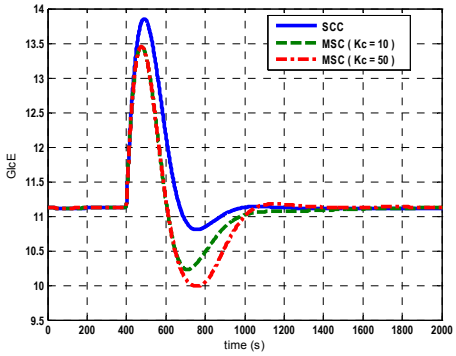

Fig. 6. Responses of scaled GlcE when subject to output disturbance of ΔEtOH = 0.1

3.2 Closed-loop Performance under GlcE Output Disturbance

The second test of control strategy performance is against the step disturbance in GlcE of 5 units (scaled unit). Fig. 7 shows the performance of both SCC strategy and MSC strategy. Clearly that MSC with Kc = 10 for micro-scale controller gives a faster response that the SCC where the former and the latter takes 3,000 seconds and 20,000 seconds respectively (more than 6 times improvement).

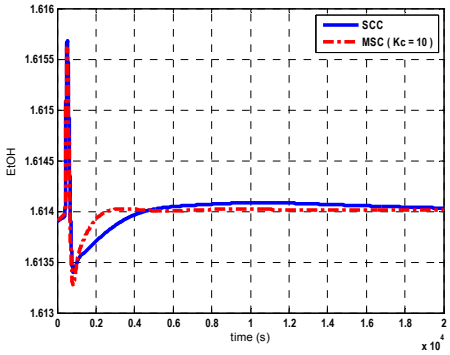

Fig. 7. Responses of scaled EtOH when subject to output disturbance of ΔGlcE = 5

Similarly, MSC strategy outperforms SCC strategy when subject to a step decrease in GlcE by 10 units. The responses of other control-loops (GlcE and L) follow the same trends as when it is subjected to EtOH disturbance i.e. does not degrade the macro-scale controller performance. Note that in both cases, the tuning of the macro-scale controllers are kept unchanged. Hence, the observed improvement is solely due to the implementation of micro-scale controller.

In addition to the step change in output disturbances, the system also has been tested against random disturbances in GlcE and EtOH. For this type of fluctuating disturbances, the frequency of the fluctuation seems to have significant effects on both SCC and MSC strategies. Due to the space limitations the results are not included in this paper.

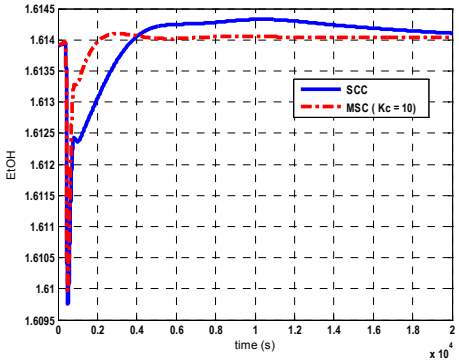

Fig. 8. Responses of scaled EtOH when subject to output disturbance of ΔGlcE = -10

4. DISCUSSIONS

Many of the potential benefits that could be harnessed from the multi-scale modeling techniques (i.e. model that embed detail knowledge at the cellular level) could not be realized due to a number of obstacles. One of these obstacles relates to the task of linking the information across the different time and length scales which is always not straightforward. Another important obstacle is the lack of analytical tools that are currently available to efficiently mine and extract vital information from such a rigorous model. As a comparison, significant benefits have been realized in the field of metabolic engineering through the applications of multi-scale models. The key reason for this success is the development of analytical tools that complement the multi-scale model, for example, metabolic control analysis (Gulik et al., 2000) has been widely applied to identify the *bottleneck* in reaction steps occurring in microbial cells.

The case study shows that a simple multi-scale control system with an appropriate control structures could significantly improve the closed-loop performance of bioreactor. The result of this paper confirms our previous study where based on idealized multi-scale system, the multi-scale control strategy can indeed lead to benefits which could not be clearly perceived from the macro-scale point of view. However, the key limitations in the application of multi-scale control strategy rest on the difficulty of identifying the suitable micro-scale inputs. In this paper, it is important to realize that NADH could not be directly manipulated in the sense of macro-scale inputs such as flowrate and fresh substrate concentration. However, NADH concentration inside the cells is generally affected by various factors such as dissolve oxygen level, pH and nutrients availability. Hence, it is still possible to indirectly manipulate the NADH by manipulating one or more of these factors. A successful example which is quite similar to this situation is the enhancement of pyruvate decarboxylase enzyme production in yeast *C. utilis* by pH adjustment (Chen et al., 2005). In metabolic engineering, one common approach in the identification of the target genes for deletion/addition is by examining the sensitivity of certain metabolites of interests to the perturbed enzymes. Then the genes expressing the enzymes which show the strong correlation with the metabolites of interest could either be

deleted or enhanced, which depends on either to minimize or maximize the metabolites respectively. So, this technique could to a certain degree be adopted in process engineering via two steps: (1) by examining the sensitivity of certain intracellular metabolites, enzymes and co-factors to the possible manipulated inputs, (2) by relating this sensitivity to the bioreactor performance. There are many possible strategies that could be explored through judicial use of multi-scale model in order to enhance the process performances as measured by yield, productivity and dynamic controllability. And in our case, simple controller algorithm with an appropriate selection of multi-scale control structure could lead to significant dynamic controllability performance. There is a reasonable ground to believe that to properly harness this knowledge it is important to develop not only the multi-scale modelling techniques but also analytical tools, which allows efficient use of the models to improving bioprocess design and operation.

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