

# Multi-scale Models for the Optimization of Batch Bioreactors

E.W.T. Liew\*, J. Nandong\* and Y. Samyudia\*<sup>§</sup>

*\*Department of Chemical Engineering, Curtin University, CDT 250, Miri 98009, Malaysia  
(E-mails: emily.liew@curtin.edu.my; jobrun.n@curtin.edu.my).*

*<sup>§</sup>Corresponding author (Tel: +6085-443835; e-mail: yudi.samyudia@curtin.edu.my)*

---

**Abstract:** In this paper, we propose multi-scale models, which are developed by expanding the so-called Herbert's Microbial Kinetics (HMK) concept so that the effects of mixing conditions are incorporated via the inclusion of the aeration rate and stirrer speed into the microbial kinetics. By using the multi-scale models, we are able to optimize the batch bioreactor's performances, i.e. yield and productivity, by adjusting the aeration rate and stirrer speed. This optimization approach is not possible using the original HMK model. Simulation and experimental studies on a batch (fermentation) bioreactor demonstrate the application of this approach, whereby the integration of the expanded HMK model with the Computational Fluid Dynamics (CFD) model of mixing, which we call it as a Kinetics Multi-Scale (KMS) model, is able to predict the experimental values of yield and productivity of the batch fermentation process accurately (with less than 5% errors).

**Keywords:** Modeling; Optimization; Batch bioreactor;

---

## 1. INTRODUCTION

Batch bioreactors are widely used in biotechnological applications, where biochemical reactions are taking place under controlled environments, i.e. temperature, oxygen, pH, nutrients and mixing conditions (Harvey and Rogers, 1996; Rahimi and Parvareh, 2005). In a fermentation process, the batch bioreactor's performances are characterized by its transport capacities in order to optimally supply the microorganism with the required nutrients and oxygen so that the metabolism occurs to produce the desired products, i.e. ethanol, and/or by products, i.e. glycerol (Lübbert, 1992)

It is very common that the batch bioreactors are equipped with impellers such that they operate in the turbulent flow regime in order to improve the mixing conditions. Controlling such mixing conditions is difficult due to the non-uniformity distribution of the intensity and quality of flow, turbulent kinetic energy, turbulent eddies and concentrations of species involved throughout the bioreactor. It is essential to devise efficient operational strategies of a bioreactor to achieve not only its good yield, but also its consistent product quality (Ranade, 1997; Venneker et al., 2002).

The common approach in the optimization of batch bioreactors has always relied on the kinetics of fermentation, which assumes well-mixing behaviour. Our previous studies, for example Liew et al. (2009) showed that the mixing behaviour could lead to severe loss in yield and changes in microbial physiology. The integration of mixing phenomena into the bioreactor modelling is therefore vital, but it is not an easy task. The detailed description of the turbulent flow field, in combination with other transport equations, needs to be addressed (Jenne and Reuss, 1999). The effects of mixing are particularly important in the case of an industrial fermentation process (Bezzo et al., 2003). It is also important to note that such a large-scale, batch fermentation process can

be highly sensitive to other variables such as batch time, liquid volume and initial nutrient concentrations as they would affect the cellular metabolism.

Process models have been utilized as tools to improve the performance of a batch bioreactor via the improvement of the metabolic capabilities of microorganism by mean of genetic manipulations of the cell metabolism and the bioprocess conditions (Wiechert, 2002). The optimisation of bioreactors now implies the manipulation of both microbial culture and the environmental conditions (Konde and Modak, 2007). Model-based optimisation is employed for systematically determining the batch operating strategies (Hjersted and Henson, 2006). Hence, process models would become more pervasive in the design, optimisation and control of bioreactors (Jiang et al., 2002).

One of the fundamental aspects to the success of the model-based optimisation of the batch bioreactors is the adopted model of microbial kinetics, which should accurately capture the effects of environmental conditions, i.e. pH, temperature, aeration rate and stirrer speed, on the rates of growth, substrate consumption and product formation. To date, most of the currently available models of microbial kinetics do not capture directly the effects of the aeration rate (AR) and stirrer speed (SS) on the rates of growth, substrate consumption and product formation.

Therefore, the main contribution of this paper lies in the development of bioreactor models, where three approaches to incorporating the effects of AR and SS into the model of microbial kinetics based on the Herbert's concepts are proposed. Two of the three developed models are referred to in this paper as the expanded Herbert's Microbial Kinetics (HMK) models, which are multi-scale in nature. The proposed approaches are not exclusively for the HMK concept. It can be extended to any other conventional model

of microbial kinetics if we want to incorporate the fluid mixing phenomena.

## 2. MATERIAL AND METHODS

In this study, a set of experiments was conducted using the BIOSTAT A-plus 2L, MO-Assembly bioreactor operated at batch mode. The industrial Baker's yeast was utilized as the inoculum culture with glucose as the substrate. The inoculum was grown in a 250mL conical flask and was incubated at room temperature for 8 hours. The 1.5L of fermentation medium was prepared by adding 75g glucose, 7.5g yeast, 3.75g NH<sub>4</sub>Cl, 4.37g Na<sub>2</sub>HPO<sub>4</sub>, 4.5g KH<sub>2</sub>PO<sub>4</sub>, 0.38g MgSO<sub>4</sub>, 0.12g CaCl<sub>2</sub>, 6.45g citric acid and 4.5g sodium citrate. The medium culture was sterilized at 121°C for 20 minutes and then cooled down to room temperature. Then, the 40mL of inoculum was added to the fermentation medium. The temperature and pH were maintained at 30°C and pH 5, respectively. The batch process was stopped after 72 hours and the samples were taken at every 2-4 hours of sampling interval and were analyzed for glucose and ethanol concentrations. The experiments were repeated at various values of AR and SS within the range of 1.0-1.5LPM of AR and 150-250rpm of SS. Each sample was first filtered, and then analyzed for the concentrations of substrate and ethanol using R-Biopharm test kits and UV spectrophotometer. All samples were tested at room temperature.

For the design experiments, we adopt the Central Composite Design (CCD). To create a CCD, it is important to locate new points along the axes of the factor space (Sampaio et al., 2010). Table 1 shows the CCD matrix employed for both the aeration rate and stirrer speed and the corresponding values of yield and productivity. For maximum efficiency, the axial or star points are to be located at a specific distance outside the original factor range. As shown in Table 1, the experimental tests involved fourteen trials. For each experimental trial, the new conditions of the aeration rate and stirrer speed were utilized.

In the application of Response Surface Methodology (Noordin et al., 2004), the regression analysis is employed to describe the experimental data collected. The least square technique is used to fit the model equation containing the input variables by minimizing the residual errors of the sum of squared deviations between the experiments and the estimated responses. These results were further analyzed by performing the ANOVA on the residuals for detecting outliers.

## 3. BIOREACTOR MODELING

The majority kinetics of ethanol fermentation utilize a formal macro-scale approach to describe the microbial growth, whereby they are empirical and based on either Monod's equation or on its numerous modifications which take into account the inhibition of microbial growth by a high concentration of product and/or substrate (Starzak et al., 1994). The models so far only explained the effect of ethanol inhibition via the mechanism of non-competitive inhibition of a simple reversible enzymatic reaction without taking into

consideration of the mixing conditions inside the batch bioreactor, i.e. the assumption of well-mixing behaviour is often applied. Deviation from the ideal mixing behaviour in practice could lead to severe loss in yield and changes in microbial physiology. Thus, the integration of mixing phenomena, i.e. the effects of AR and SS, is necessary taken into account, and this is the objective of our modelling work.

Table 1. CCD matrix for the two independent variables (Aeration Rate and Stirrer Speed)

Standard Order	Run Order	Block	X <sub>1</sub> :Aeration Rate (LPM)	X <sub>2</sub> :Stirrer Speed (rpm)	Y <sub>1</sub> :Yield (%)	Y <sub>2</sub> : Productivity (g/L.hr)
7	1	1	1.25	200	21.500	0.180
1	2	1	1.0	150	14.788	0.099
5	3	1	1.25	200	21.050	0.176
6	4	1	1.25	200	21.250	0.178
3	5	1	1.0	250	15.105	0.102
4	6	1	1.5	250	24.040	0.160
2	7	1	1.5	150	16.392	0.106
13	8	2	1.25	200	24.000	0.230
12	9	2	1.25	200	23.500	0.200
14	10	2	1.25	200	22.000	0.190
10	11	2	1.25	129.29	18.511	0.115
9	12	2	1.60	200	22.250	0.195
11	13	2	1.25	270.71	23.500	0.210
8	14	2	0.90	200	20.500	0.165

Three modelling approaches to incorporating the effects of mixing in a batch ethanolic fermentation process are therefore proposed in this paper: (1) statistical data-based (SDB) model, (2) kinetics hybrid (KH) model and (3) kinetics multi-scale (KMS) model. To validate the developed models, a series of experimental studies (as explained in Section 2) were conducted.

### 3.1 Kinetics Herbert's concept

In order to predict the yield  $Y$  and productivity  $Pr$ , a batch bioreactor can be modelled dynamically as follows:

$$\dot{Z} = \begin{bmatrix} \dot{X}_v \\ \dot{S} \\ \dot{P} \end{bmatrix} = \begin{bmatrix} r_x \\ r_s \\ r_p \end{bmatrix} \quad (1)$$

$$\Phi = \begin{bmatrix} Y \\ Pr \end{bmatrix} = \begin{bmatrix} 100P(t_b)/(S_o - S(t_b)) \\ (P(t_b) - P_o)/t_b \end{bmatrix} \quad (2)$$

where  $S_o = S(0)$  and  $P_o = P(0)$  is the initial substrate and ethanol concentrations (g/L) of the medium,  $t_b$  (hrs) is the batch time for the fermentation process. Other variables are the concentration profiles of substrate  $S$ , product (ethanol)  $P$  and viable cell (biomass)  $X_v$ . The microbial kinetics are: (1) rate of growth  $r_x$ , (2) rate of product formation  $r_p$  and (3) rate of substrate consumption  $r_s$ .

In this work, the Herbert's concept of endogenous metabolism is adopted since it has been used in numerous studies to describe the kinetics of ethanolic fermentation with sufficient accuracy (Starzak et al. 1994). Moreover, the by-product concentration is not included in this study because

we focus on the impacts of AR and SS on the concentration profiles of substrate  $S$ , product (ethanol)  $P$  and viable cell (biomass)  $X_v$ . The Herbert's concept assumes that the observed rate of biomass formation  $r_x$  comprised of the growth rate  $(r_x)_g$  and the rate of endogenous metabolism  $(r_x)_{end}$ :

$$r_x = (r_x)_g + (r_x)_{end} \quad (3)$$

Where:

$$(r_x)_g = [k_1 X_v S / (k_2 + S)] \exp(-k_5 P) \quad (4)$$

$$(r_x)_{end} = -k_6 X_v \quad (5)$$

The rates of substrate consumption and product formation are assumed to be proportional to the biomass growth rate:

$$r_s = -k_3 (r_x)_g \quad (6)$$

$$r_p = k_4 (r_x)_g \quad (7)$$

Note that the kinetics of ethanolic fermentation based on the Herbert's concept consists of six kinetic parameters i.e.  $\kappa = [k_1 \ k_2 \ k_3 \ k_4 \ k_5 \ k_6]^T$ .

whereby the mixing effects in terms of AR and SS is to be included in each kinetic parameters.

### 3.2 Statistical Data-Based (SDB) Model

Rather than using the bioreactor models from (1) to (7), the SDB model is developed by applying a regression analysis to a set of experimental data for different aeration rates (AR), stirrer speed (SS), yields ( $Y$ ) and productivity ( $Pr$ ). By applying this approach, the effect of mixing arising from different values of aeration rate (AR) and stirring speed (SS) is included in the regression model by treating the AR and SS as inputs (or experimental variables  $X$  and the yield  $Y$  and productivity ( $Pr$ ) as outputs (or response variables  $\Phi$ ). After applying design experiments to the bioreactor, sets of experiment data for both inputs and outputs are obtained.

Generally in the development of statistical-based model, it is assumed that there exists a relationship  $F: X \rightarrow \Phi$  where  $X \in \mathfrak{R}^n$  is an  $n$ -dimensional vector of inputs,  $\Phi \in \mathfrak{R}^m$  is an  $m$ -dimensional vector of outputs and  $F \in \mathfrak{R}^m$  is an  $m$ -dimensional vector of functions space. As  $\Phi$  is normally an implicit function of  $X$ , in real practice it is often difficult to obtain the exact relationship between the input and response vectors especially for complex systems. Thus, one way to develop an explicit relationship between them is by using a regression model where the model parameters are obtained by an optimisation.

Let us consider the regression model as a quadratic model:

$$\hat{\Phi} = A + BX + X^T DX + \varepsilon \quad (8)$$

where  $A, B, D$  are defined as model parameters, whereby  $A, B$  and  $D$  will be estimated in such a way that the sum of the

squared errors between the predicted values  $\hat{\Phi}$  and experimental values  $\Phi$  of the responses are minimised. So, this problem can be mathematically stated as:

$$P_1: \min_{A,B,D} \left\{ \sum_{i=1}^k [(\hat{\Phi}_i - \Phi_i)]^T [(\hat{\Phi}_i - \Phi_i)] \right\} \quad (9)$$

Where  $\hat{\Phi}_i$  is the predicted values by (8) and the subscript  $i$  indicates the experimental number. Based on the full factorial design, the total number of experimental runs for  $n$  inputs is given by  $k = 2^n$ ; in our case  $n = 2$  and hence  $k = 4$ . Note that different values of  $k$  result in different experimental designs.

To test the optimisation results of  $P_1$ , the Analysis of Variance (ANOVA) is performed where the results must be significant along with the analysis of curvature. Curvature analysis is vital to indicate whether the experimental results could fit well into the proposed model (Wadsworth, 1998). If the curvature is significant, i.e. the curvature lies in the region of the desired optimum response, the optimisation results are acceptable. If the curvature is insignificant, the optimisation results are not acceptable. A method based on the Path of Steepest Ascent (PSA) (Wadsworth, 1998) is adopted, and where the curvature is further analyzed until it is shown to be significant.

Note that some process constraints such as  $X_{\min} \leq X \leq X_{\max}$  where  $X_{\min}$  and  $X_{\max}$  denote the lower and upper limits of inputs, respectively can be included in the optimisation of  $P_1$ . Furthermore, different model structures can also be selected while solving the optimisation such as linear or second order model as:

$$\Phi = A + BX + \varepsilon, \text{ or} \quad (10)$$

$$\Phi = A + BX + XX^T C^T + \varepsilon \quad (11)$$

Where  $C$  is an off-diagonal matrix defined as:  $C = \begin{bmatrix} 0 & c_1 \\ c_2 & 0 \end{bmatrix}$ .

### 3.3 Kinetics Hybrid (KH) Model

The basic assumption underlying the development of Kinetics Hybrid model is that the kinetic parameters are the function of the inputs  $X$  as:

$$\kappa = h(X, \theta) \quad (12)$$

Here  $\theta \in \mathfrak{R}^{n_\theta \times m_\theta}$  is a matrix whose elements correspond to the parameters to be determined later. Therefore substituting (12) into (3) to (7), the microbial kinetics of Herbert's can now be expressed as:

$$R_x = g_m(Z, X, \theta) \quad (13)$$

The advantage of expanded Kinetics Herbert's model, i.e. (13), over the original microbial kinetic model, i.e. (3) to (7) is that the expanded one can be directly used to optimize the

yield  $Y$  and the productivity  $Pr$  with respect to the aeration rate (AR) and stirrer speed (SS).

The development of Kinetics Hybrid model involves two main steps:

1. For experimental run  $i$ , obtain the kinetics parameters  $\kappa_i$  using the original kinetics Herbert's model based on (3) to (7) and batch reactor model based on (1) to (2).
2. For the obtained kinetic parameters  $\kappa_i, \forall i \in \{1, 2, \dots, k\}$  and sets of aeration rates (AR <sub>$i$</sub> ) and stirrer speeds (SS <sub>$i$</sub> ), find  $\theta$  in (12) using regression method.

The combination of the batch bioreactor model which is denoted by (1) to (2), the Herbert's kinetics model, denoted by (3) to (7) and the regression model of (12) constitutes the so-called kinetics hybrid (KH) model of bioreactor. Clearly, in this approach, the effect of mixing is now embedded into the bioreactor model.

In more details, the development of kinetics hybrid model follows the systematic procedure as:

#### Step 1: Identification of Herbert's Kinetic Parameters

The Herbert's kinetic parameters ( $k_1, k_2, \dots, k_6$ ) are obtained via optimisation by solving the following quadratic problem:

$$P_2 : \min_{\kappa} \left\{ \sum_{j=1}^s [Z_j - \hat{Z}_j]^T [Z_j - \hat{Z}_j] \right\} \quad (14)$$

Here  $\hat{Z}_j$  is the predicted value of  $Z$  using the bioreactor model of (1) to (2) at the  $j$ -sample time, and  $s$  is the number of samples taken during the course of batch experiments.

For the  $i$ -experimental run, the corresponding solution to problem  $P_2$  will yield  $\kappa_i = \kappa_i^*$  that minimizes the sum of squared errors between the predicted values and experimental values of  $Z_i$ . For a  $k+1$  number of experimental runs, we will obtain a set of  $\kappa = \{\kappa_o^*, \kappa_1^*, \kappa_2^*, \dots, \kappa_k^*\}$ , which contains the solutions corresponding to all experimental runs including the base-line experimental run i.e.  $\kappa_o^*$ .

#### Step 2: Determine the Regression Model of (12)

This step is to find the regression model of  $\kappa$  in term of  $X$ . Thus, this is equivalent to finding  $\theta = \theta^*$  where the problem can be stated as:

$$P_3 : \min_{\theta} \left\{ \sum_{i=0}^j [\kappa_i - \hat{\kappa}_i]^T [\kappa_i - \hat{\kappa}_i] \right\} \quad \forall \kappa_i \in \kappa \quad (15)$$

where  $j$  is the number of experiment runs based on the design of experiment (i.e.  $j=4$  if factorial design is adopted). Here  $i=0$  indicates the experiment at the base-line conditions and  $\hat{\kappa}$  denotes the predicted value of  $\kappa$  based on a regression model, e.g. as (8), (10) or (11). As we have no a priori knowledge on the exact form of relationships  $h: X \rightarrow \kappa$ , we use the statistical approach, i.e. the technique used for the SDB model, thus assuming  $\kappa$  can be represented by model equations e.g. by (10) for linear model.

### 3.4 Kinetics Multi-Scale (KMS) Model

The kinetics multi-scale (KMS) model is developed based on the Kinetics Hybrid model described by (3) to (7) and (9) to (10), but we use the general mass-energy balance over an element of reactor volume combined with a mixing model to replace the bioreactor model which is denoted by (1) to (2). The mixing model is implemented using Computational Fluid Dynamics (CFD) based on the  $k$ - $\varepsilon$  turbulence model (Dubey et al., 2006). This approach was used to describe the mixing mechanism in a bioreactor with sufficient accuracy (Ranade, 2002).

The  $k$ - $\varepsilon$  turbulence model is normally used in order to describe the mixing behaviour and to compute the turbulence in the bioreactor. The energy dissipation is expressed as:

$$\varepsilon = ((\Delta P F u)) / m = (\Delta P u) / (x \rho) \quad (16)$$

where  $\Delta p$  denotes the pressure drop,  $m$  the mass,  $F$  the tube cross-section,  $x$  the axial coordinate and  $\rho$  the density of the fluid. Moreover, the fluid flow equations need to be solved for a constant density fluid (Bode, 1994). These consist of the continuity equation:

$$\text{div}(\rho u) = 0 \quad (17)$$

and the transport equations:

$$\text{div}(\rho u k) = \text{div}[(\mu_{eff} / \sigma_k) \nabla k] + G - \rho \varepsilon \quad (18)$$

$$\text{div}(\rho u \varepsilon) = \text{div}[(\mu_{eff} / \sigma_\varepsilon) \nabla \varepsilon] + (C_1 G - C_2 \rho \varepsilon) (\varepsilon / k) \quad (19)$$

where  $\nabla k$  and  $\nabla \varepsilon$  denote the gradient of  $k$  and  $\varepsilon$ , respectively.  $k$  is the kinetic energy of turbulence at the point of interest.

The Eddy Viscosity is given by:

$$u_T = C_\mu \rho k^2 / \varepsilon \quad (20)$$

Note that  $G$  is the scalar dissipation function ( $G = \tau_{ij} \tau_{ij} / 2 \mu_{eff}$ ) and the scalar values:  $C_\mu = 0.09$ ,  $C_1 = 1.44$ ,  $C_2 = 1.92$ ,  $\sigma_k = 1$  and  $\sigma_\varepsilon = 1.3$ .

The Navier-Stokes equation is used for flow equations to describe the instantaneous behaviour of the turbulent liquid flow in ethanolic fermentation process. The resulting Reynolds equations and the continuity equation are given by:

$$\frac{\partial(\rho u_i)}{\partial t} + \frac{\partial(\rho u_i u_j)}{\partial x_j} = -\frac{\partial}{\partial x_i} (\tau_{ij} + \rho u_i u_j) - \frac{\partial p}{\partial x_i} + \rho g_i \quad (21)$$

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x_i} (\rho u_i) = 0 \quad (22)$$

For model accuracy and computational expense, a reasonable eddy viscosity models relating the individual Reynolds stresses to mean flow gradients are adopted:

$$\rho u_i u_j = -\rho \nu_{turb} \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) + \frac{2}{3} \rho \delta_{ij} k \quad (23)$$

where  $\nu_{turb}$  is the turbulent eddy viscosity. The transport of momentum is thought of as turbulent eddies, which like molecules, collide and exchange momentum.

The general balance over an element of reactor volume is given by:

$$\partial(\rho\phi)/\partial t + \partial(\rho U_i \phi)/\partial x_i = \partial(\Gamma_\phi(\partial\phi)/\partial x_i)/\partial x_i + S_\phi \quad (24)$$

where  $\rho$  is the density of fluid,  $\phi$  is the concentration of any component,  $U_i$  is the local velocity in the  $x_i$ -direction,  $\Gamma_\phi$  is the effective diffusivity of  $\phi$  and  $S_\phi$  is the volumetric source term (rate of production of  $\phi$  per unit volume). Note that, the reaction rates described by (13) are embedded into the source term  $S_\phi$ . Also note that the notation  $\phi = Z$  denotes the output variables i.e. biomass, substrate and ethanol concentrations. Using the KMS model, we can compute the mass of substrate and mass of product at the end of batch time over the total reactor volume, i.e.:

$$MS_{t_b} = \sum_{i=1}^h (S_i \Delta V_i) \quad (25)$$

$$MP_{t_b} = \sum_{i=1}^h (P_i \Delta V_i) \quad (26)$$

where the volume of medium in the reactor  $V_R$  is given by:

$$V_R = \sum_{i=1}^h \Delta V_i \quad (27)$$

Here  $h$  is the number of discretizations (i.e. finite volume). Then, we can calculate as follows:

$$\Phi_\theta = \begin{bmatrix} Y \\ Pr \end{bmatrix} = \begin{bmatrix} 100(MP_{t_b} - MP_o)/(MS_o - MS_{t_b}) \\ (MP_{t_b} - MP_o)/t_b \end{bmatrix} \quad (28)$$

Here  $MS_o$  and  $MP_o$  correspond to the initial mass of substrate and mass of product (ethanol) in the fermentation medium, respectively.

### 3.5 Model Analysis and Validation

In this section, we develop the models using the proposed approaches, then the models are analysed using Analysis of Variance (ANOVA) and validated against the experimental data (Noordin et al., 2004). The SDB model was developed using the input and output data as shown in Table 1, and the regression method results in the following quadratic model (29) where the ANOVA analysis is presented in Tables 2 and 3:

$$\begin{bmatrix} Y \\ Pr \end{bmatrix} = \begin{bmatrix} 33.098 \\ 0.234 \end{bmatrix} + \begin{bmatrix} -18.785 & -0.143 \\ -0.139 & -9.9 \times 10^{-4} \end{bmatrix} \begin{bmatrix} AR \\ SS \end{bmatrix} + \begin{bmatrix} AR & SS \end{bmatrix} \begin{bmatrix} 0 & 0.147 \\ 1.02 \times 10^{-3} & 0 \end{bmatrix} \begin{bmatrix} AR \\ SS \end{bmatrix} \quad (29)$$

Table 2. ANOVA Results for Yield

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	P-value Prob > F		
Model	90.10	5	18.02	9.73	0.0047	Sig.	
A - AR	21.17	1	21.17	11.44	0.0117		
B - SS	28.20	1	28.20	15.24	0.0059		
AB	13.44	1	13.44	7.26	0.0309		
A <sup>2</sup>	12.76	1	12.76	6.90	0.0341		
B <sup>2</sup>	16.60	1	16.60	8.97	0.0201		
Residual	12.96	7	1.85				
Lack of Fit	10.64	3	3.55	6.12	0.0563		Not Sig.
Pure Error	2.32	4	0.58				
Cor Total	132.31	13					

Table 3. ANOVA Results for Productivity

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	P-value Prob > F		
Model	0.015	5	2.986E-3	9.30	0.0054	Sig.	
A - AR	1.443E-3	1	1.443E-3	4.49	0.0718		
B - SS	4.577E-3	1	4.577E-3	14.25	0.0069		
AB	6.503E-4	1	6.503E-4	2.02	0.1978		
A <sup>2</sup>	2.868E-3	1	2.868E-3	8.93	0.0203		
B <sup>2</sup>	5.981E-3	1	5.981E-3	18.62	0.0035		
Residual	2.248E-3	7	3.212E-4				
Lack of Fit	1.369E-3	3	4.563E-4	2.08	0.2461		Not Sig.
Pure Error	8.793E-4	4	2.198E-4				
Cor Total	0.024	13					

Table 4. Predicted Herbert's Kinetics Parameters

Run Order	$X_f$ : AR (LPM)	$X_2$ : SS (rpm)	$\hat{k}_1$	$\hat{k}_2$	$\hat{k}_3$	$\hat{k}_4$	$\hat{k}_5$	$\hat{k}_6$
1	1.25	200	1.4085	0.0010	0.6631	0.1040	0.7558	0.0143
2	1.0	150	1.3245	0.0010	0.6559	0.0770	0.8788	0.0163
3	1.25	200	1.1257	0.0010	0.6533	0.0909	0.7252	0.0173
4	1.25	200	1.2591	0.0010	0.6731	0.0879	0.7127	0.0179
5	1.0	250	2.0629	0.0010	0.6999	0.1026	0.8366	0.0125
6	1.5	250	1.4925	0.0010	0.6703	0.1310	0.6328	0.0123

As shown in Tables 2 and 3, the ANOVA results of the model demonstrate that the model is highly significant, as indicated by the Fisher's  $F$  test ( $F_{model} = 9.73$  and  $9.3$ ) and a low "Prob > F" value ( $P_{model} > 0.0047$  and  $> 0.0054$ ). Additionally, the goodness of the fit of the model is also checked by the determination coefficient ( $R^2$ ). In this case, the values of the determination coefficient ( $R^2 = 0.8743$  and  $R^2 = 0.8691$ ) indicate that 87% of the sample variation in yield and productivity are well explained by the model. Thus, the SDB model is statistically adequate to predict the yield and productivity within the range of experimental setting.

For the kinetics hybrid model, solving the optimisation problem of  $P_2$  gives the predicted Herbert's kinetic parameters as shown in Table 4. This set of data is then used to obtain the following regressed linear model by solving the optimisation problem of  $P_3$ :

$$\begin{bmatrix} k_1 \\ k_2 \\ k_3 \\ k_4 \\ k_5 \\ k_6 \end{bmatrix} = \begin{bmatrix} 1.4085 - 0.2852X_1 + 0.3692X_2 \\ 0.0010 \\ 0.6631 - 0.0148X_1 + 0.0220X_2 \\ 0.1040 + 0.0142X_1 + 0.0128X_2 \\ 0.7558 - 0.1019X_1 - 0.0211X_2 \\ 0.0143 - 0.0001X_1 - 0.0019X_2 \end{bmatrix} \quad (30)$$

By combining the linear model of (30) with the macro scale bioreactor of (1) to (7), the kinetics hybrid model was obtained. This model is validated against another set of experimental data of AR and SS, which were chosen within the experimental range, i.e. 1.2 LPM AR and 175rpm SS. Fig. 1 to Fig. 2 show the example results of model validation. As observed, the kinetics hybrid model reasonably fits the experimental data.

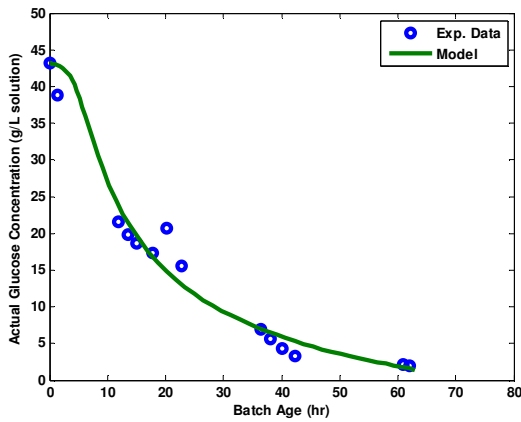


Fig. 1. Model fitting for actual glucose concentration (g/L solution) vs batch age (hr)

Similarly, a series of simulations were performed to validate the KMS model, where a set of AR and SS was generated using the CCD technique. The ANOVA results of the fitness of the KMS model are shown in Tables 5 and 6.

The determination coefficients of the KMS model are  $R^2 = 0.8353$  and  $R^2 = 0.8021$ , respectively for the yield and productivity. This result shows that more than 80% of the sample variation in yield and productivity are well explained by the model. Thus, statistically the model is sufficiently accurate in term of the prediction for yield and productivity within the experimental range.

### 3.6 Optimization of bioreactor's performances

#### 3.6.1. Problem Formulation

Our objective is to find the optimum AR and SS by maximizing the yield and productivity using the developed models, i.e. SDB, KH and KMS models. The optimisation problem is formulated as follows:

$$P_4 : \max_{X_{\min} \leq X \leq X_{\max}} \{(\phi^T \phi)\} \quad (31)$$

Subject to: the model (either SDB, KH or KMS Model).

To solve this optimisation problem, we can use a nonlinear programming technique, but in this study, we employ the Response Surface Methodology (RSM) technique to find the optimum AR and SS (Sampaio et al., 2010).

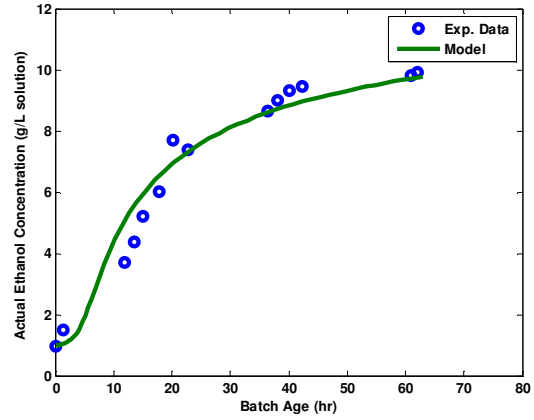


Fig. 2. Model fitting for actual ethanol concentration (g/L solution) vs batch age (hr)

Table 5. ANOVA results on yield for the KMS model

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	Sig.
Model	52.65	5	10.53	7.10	0.0115	Sig.
Residual	10.38	7	1.48			
Lack of Fit	6.75	3	2.25	2.48	0.2003	Not Sig.
Pure Error	3.63	4	0.91			

Table 6. ANOVA Results on productivity for the KMS model

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	Sig.
Model	0.016	5	3.144E-3	4.74	0.0328	Sig.
Residual	4.641E-3	7	6.630E-4			
Lack of Fit	3.159E-3	3	1.053E-3	2.84	0.1693	Not Sig.
Pure Error	1.481E-4	4	3.703E-4			

#### 3.6.2. Simulation Results

After applying the RSM technique, a number of 3D surface plots were generated for finding the optimum AR and SS using the SDB model as shown in Fig. 3. From Fig. 3, we observe that there is a significant (quadratic) effect of AR and SS on the response surface. Within the experimental range, the optimum values of AR and SS maximizing yield and productivity are **1.47LPM** and **242rpm**, respectively. The maximum values of yield and productivity are **24.5%** and **0.2g/L.hr**, respectively. This value of yield corresponds to 97.8% of the maximum theoretical value for yield of ethanol. In general, the response of yield increases as the SS increases from 150rpm to its peak value at 242rpm. Additionally, the yield shows a significant increase with the increase in AR. Overall, the SDB model demonstrates a reasonable prediction

of the impacts of AR and SS on the values of yield and productivity.

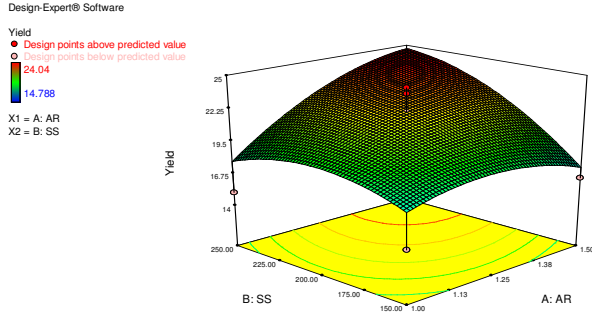


Fig. 3. Response surface plots for the SDB Model

For the KH model, the effects of AR and SS on the yield and productivity are shown in Fig. 4. The surface responses show that both yield and productivity are significantly affected by AR and SS. Also, it can be seen that the yield and productivity increase with the increase of AR and SS. Thus, this suggests that the KH model was able to capture the effect of both AR and SS on the yield and productivity. Just like the SDB model, the KH model is able to predict the impacts of AR and SS on the yield and productivity reasonably well.

The optimum values of AR and SS were obtained using the KH model as **1.43LPM** and **250rpm**, respectively. These optimum values lead to the **maximum yield of 21.150%** and **maximum productivity of 0.150g/L.hr**. Therefore, the maximum yield corresponds to 96.6% of the maximum theoretical value for yield. Also, we note that the predicted maximum yield and productivity by the KH model are comparable with that of the SDB model.

Fig. 5 shows the response surface plots for the KMS model. Like other models, the KMS model shows that the yield and productivity increase with the increase of AR and SS. Within the experimental range, the optimum values of AR and SS are **1.45LPM** and **240rpm**, respectively. These values correspond to the **maximum yield of 24.128%** and **maximum productivity of 0.207g/L.hr**. It is interesting to note, though, that the response surface plots generated based on the KMS model are almost similar to those generated by the SDB model. In contrast, the response surface plots of the KMS model are quite different from those of the KH model. This is due to the assumption of well-mixing behaviour in the macro bioreactor model used in the KH model.

### 3.6.3. Experimental Validation

In this section, we present the experimental validation of the optimum values of AR and SS found using different models. Tables 7 and 8 show the prediction errors of the maximum yield and productivity using different models. The errors were calculated as:

$$Error Yield = 100\% \times |Yield_{max} - Yield_i| / Yield_{max} \quad (32)$$

$$Error Pr od = 100\% \times |Pr od_{max} - Pr od_i| / Pr od_{max} \quad (33)$$

where the subscript  $i$  refers to the  $i$ -model, i.e.  $i \in \{SDB, KH, KMS\}$ .

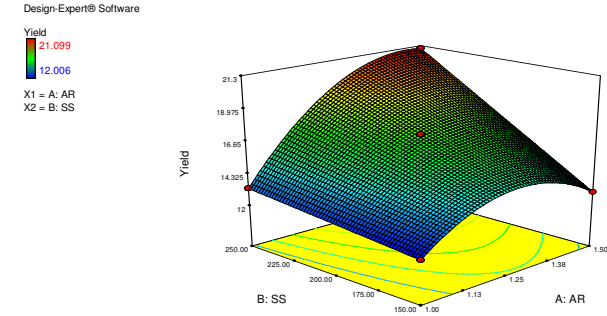


Fig. 4. Response surface plots for the KH Model

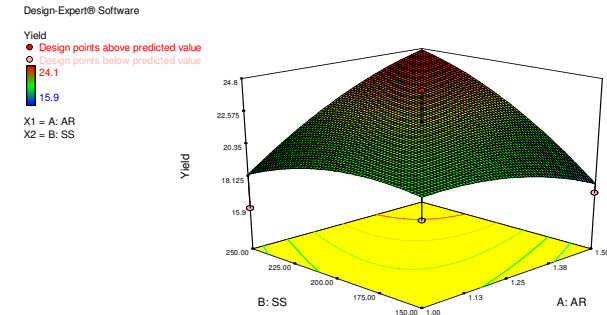


Fig. 5. Response surface plots for the KMS Model

Note that the KMS model exhibits the best prediction of maximum experimental yield and productivity. Despite its simplicity, the SDB model predictions are relatively good and better than the KH model predictions. This means that by including the CFD model in the macro bioreactor model, the effect of mixing arising from the AR and SS can reasonably be captured by the KMS model – provide the most accurate prediction of yield and productivity.

Meanwhile, the KH model is only capable of taking into account the effect of AR and SS within the context of well-mixing condition inside the bioreactor. The fact that KH model resulted in the largest error in the predictions of maximum experimental yield and productivity suggested that there was a significant deviation from ideal mixing inside the bioreactor. Interestingly, despite this significant deviation from the ideal mixing condition, the SDB model, which directly expressed the yield and productivity as a quadratic function of AR and SS, was shown to be capable of predicting the maximum yield and productivity with a sufficient accuracy i.e. less than 5% error.

Table 7. Comparisons of Model Predictions and Experimental Results for Yield

<i>i</i> -Model	Optimum Aeration Rate (LPM)	Optimum Stirrer Speed (rpm)	Model Predicted Maximum Yield (%)	Experimentally Verified Maximum Yield (%) $Yield_i$	Error Yield (%)
SDB	1.47	242	24.495	23.720	3.46
KH	1.43	250	21.150	20.950	14.73
KMS	1.45	240	24.128	24.570 <sup>l</sup>	1.80

<sup>l</sup> The yield obtained by using the AR and SS values from the KMS model is adopted as the experimentally maximum value, with respect to which the error is calculated.

Table 8. Comparisons of Model Predictions and Experimental Results for Productivity

<i>i</i> -Model	Optimum Aeration Rate (LPM)	Optimum Stirrer Speed (rpm)	Model Predicted Maximum Prod. (g.L/hr)	Experimentally Verified Maximum Prod. (g.L/hr) $Prod_i$	Error Prod. (%)
SDB	1.47	242	0.198	0.185	11.90
KH	1.43	250	0.150	0.148	29.52
KMS	1.45	240	0.207	0.210 <sup>l</sup>	1.43

<sup>l</sup> The productivity obtained by using the AR and SS values from the KMS model is adopted as the experimentally maximum value, with respect to which the error is calculated.

#### 4. CONCLUSIONS

In this paper, we have proposed three multi-scale models of a batch bioreactor by expanding the Herbert's kinetics concept to incorporate the mixing conditions. It was shown that the models could be used to optimize the bioreactor's performances. Furthermore, it was found from this study that the incorporation of mixing CFD model into the KH model of microbial kinetics (i.e. KMS modelling approach) could predict reasonably well the optimum yield and productivity by adjusting the aeration rate and stirrer speed. As the results, the developed models could be used for studying the effects of AR and SS on the rates of growth, substrate consumption and product formation, which is not possible using the conventional models.

#### ACKNOWLEDGEMENTS

The work described in this paper was supported by the eScienceFund project (No: 02-02-07-SF0001) under the Ministry of Science, Technology and Innovation (MOSTI).

#### REFERENCES

Bode, J. (1994). Computational fluid dynamics applications in the chemical industry. *Computers chem. Engng*, **18**, S247-S251.

Bezzo, F., S. Macchietto, C. C. Pantelides (2003). General hybrid multizonal/CFD approach for bioreactor modeling. *AIChE Journal*, **49**, 2133-2148.

Dubey, H., S. K. Das, T. Panda (2006). Numerical simulation of a fully baffled biological reactor: The differential

circumferential averaging mixing plane approach. *Biotechnology and Bioengineering*, **95**, 754-766.

Harvey, A.D., S. E. Rogers (1996). Steady and unsteady computation of impeller-stirred reactors, *AIChE Journal*, **42**, 2701-2712.

Hjersted, J.L., M. A. Henson (2006). Optimization of fed-batch *Saccharomyces cerevisiae* fermentation using dynamic flux balance models, *Biotechnol. Prog.*, **22**, 1239-1248.

Jenne, M., M. Reuss (1999). A critical assessment on the use of k-e turbulence model for simulation of turbulent flow induced by a Ruston turbine in baffled stirred tank reactor. *Chem. Eng. Sci.*, **54**, 3921-3941.

Jiang, Y., M. R. Khadilkar, M. H. Al-Dahhan, M. P. Dudukovic (2002). CFD of multiphase flow in packed-bed reactors: II. results and applications, *AIChE Journal*, **48**, 716-730.

Konde, K.S., J. M. Modak (2007). Optimization of bioreactor using metabolic control analysis approach. *Biotechnol. Prog.*, **23**, 370-380.

Liew, E.W.T., J. Nandong, Y. Samyudia (2009). Experimental investigation on the impact of aeration rate and stirrer speed on micro-aerobic batch fermentation. *Journal of Applied Sciences*, **9**, 3126-3130.

Lübbert, A. (1992). Advanced methods for bioreactor characterization. *Journal of Biotechnology*, **25**, 145-182.

Noordin, M.Y., V. C. Venkatesh, S. Sharif, S. Elting, A. Abdullah (2004). Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology*, **145**, 46-78.

Rahimi, M., A. Parvareh (2005). Experimental and CFD investigation on mixing by a jet in a semi-industrial stirred tank. *Chemical Engineering Journal*, **115**, 85-92.

Ranade, V.V. (1997). An efficient computational model for simulating flow in stirred vessels: a case of Rushton turbine. *Chemical Engineering Science*, **52**, 4473-4484.

Ranade, V.V. (2002). *Computational flow modeling for chemical reactor engineering*. Process Systems Engineering, UK.

Sampaio, P.N., C.R.C. Calado, L. Sousa, D.C. Bressler, M.S. Pais, L.P. Fonseca (2010). Optimization of the culture medium composition using response surface methodology for new recombinant cyprosin B production in bioreactor for cheese production. *Eur Food Res Technol*, **231**, 339-346.

Starzak, M., L. Krzystek, L. Nowicki, H. Michalski (1994). Macroapproach kinetics of ethanol fermentation by *Saccharomyces cerevisiae*: experimental studies and mathematical modelling. *The Chemical Engineering Journal*, **54**, 221-240.

Venneker, B.C.H., J. J. Derksen, H. E. A. Van den Akker (2002). Population balance modeling of aerated stirred vessels based on CFD. *AIChE Journal*, **48**, 673-685.

Wadsworth, H.M. (1998). *Handbook of Statistical Methods for Engineers and Scientists, second ed.* McGraw-Hill.

Wiechert, W. (2002). Modeling and simulation: tools for metabolic engineering. *Journal of Biotechnology*, **94**, 37-63.