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# Computational F*luid* Dynamics of Mixing in Aerated Bioreactors

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***Abstract*—In this paper, we address a kinetics multi-scale bioreactor model in terms of aeration rate and stirrer speed to describe the mixing phenomena in an aerated bioreactor. Aeration rate and stirrer speed are chosen as factors to investigate whether both parameters will aid in the prediction of the mixing phenomena, yield and productivity of the bioreactor. It is vital to do so since improper mixing will deteriorate the yield and productivity of the bioreactor due to the multi-scale operation of the bioreactor. The developed kinetics model is verified on a 0.002m$^3$ bioreactor equipped with a centrally located six-blade Rushton turbine impeller. Combined analysis of the experimental results with the modeling of flow field using Computational Fluid Dynamics (CFD) showed that aeration rate and stirrer speed are factors affecting the fermentation process and aid in the mixing mechanism in the bioreact*or*.**

***Keywords-aeration rate; CFD; mixing; stirrer speed***

## I. INTRODUCTION

Bioreactor has been recognized as the heart of biotechnological processes which provide central link between raw materials and products [1]. The bioreactor operation is multi-scale, whereby cellular level consists of numerous biochemical reactions catalyzed by thousands of enzymes. In order to achieve effective and efficient bioreactor performance, it is vital to ensure good mixing is induced so that optimal conditions can be maintained throughout the bioreactor [2]. The key objectives of mixing are to overcome transport phenomena limitations and to homogenize the conditions inside the bioreactor, i.e. to avoid dead zones. Conventional control tries to induce good mixing but an ideal (i.e. one that is well-mixed and free of disturbances) operation is difficult to achieve, thus recent work has tried to develop the non-ideal features to improve performance [3]. It is generally assumed that an ideal bioreactor is most favorable to cell growth and product formation. However, it has been recognized that real operations are inevitably deviate from ideal behavior. It is impractical to set ideal operation as the objective due to the limitations of mixing devices and measuring instruments (including cost) [4] and the weaknesses of noise filtering methods [3] make it tough to achieve perfect mixing. Thus, the interaction between mixing and chemical reactions has attracted much attention over the last decades.

Over the decade, numerous research models have been published to describe the kinetic behavior of alcoholic fermentation [5]. Lack of suitable models and general complexity of bioreactors are major obstacles in numerical simulations [6]. One of the most challenging tasks is the design of highly shear-thinning viscous fermentation broths, where the main limiting factors are bulk mixing and oxygen mass transfers. With Computational Fluid Dynamics (CFD), it is possible to model these conditions in arbitrary vessel geometries since CFD has been used for modeling mixing problems.

In this work, CFD simulated results for yield and productivity were compared with experimental data in a certain range of operating conditions, varying both aeration rate and stirrer speed of the impeller in order to investigate the mixing profile of the aerated bioreactor. A kinetics multi-scale model or non-ideally mixed kinetics model is proposed and will be implemented into CFD simulation in order to investigate the effect of yield and productivity as well as the mixing profile of the fermentation process under different conditions of both aeration rate and stirrer speed. Although there are many models available in the field of alcoholic fermentation, our interest is centered on non-ideally mixed models.

## II. MODELING APPROACH

The majority modeling approach of alcoholic fermentation utilize a formal (macro) approach in order to describe the microbial growth based on either Monod's equation or on its numerous modifications which take into account the inhibition of microbial growth by a high substrate/product concentration [7]. So far, models proposed deviate from ideal mixing behavior, without considering the mixing mechanism which could lead to severe loss in yield and changes in microbial physiology [8]. Thus, in order to describe the mixing phenomena, a kinetics multi-scale model is proposed based on Herbert's concept of endogenous metabolism (kinetics model), macro-scale bioreactor model and mixing model [9]. All of these models will be combined and will be implemented into CFD simulations so as to investigate the mixing mechanism within the stirred bioreactor. Aeration rate (*AR*) and stirrer speed (*SS*) will be implemented into the proposed model in

order to investigate whether these variables will affect the mixing mechanism.

Fig. 1 shows the schematic diagram of the proposed kinetics multi-scale model with the implementation of both *AR* and *SS*.

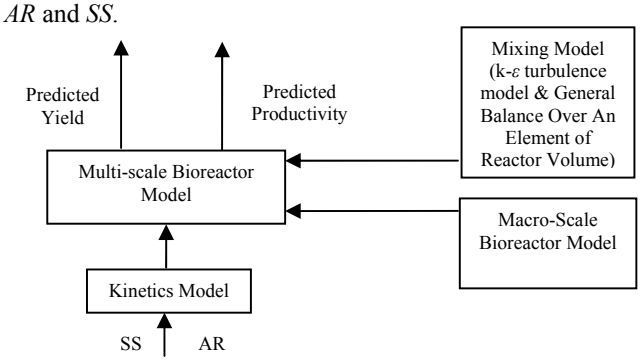


Figure 1. Schematic Diagram of Kinetics Multi-Scale Model.

*A. Herbert's Kinetics Model*

Herbert's concept is chosen since it has been used in numerous studies to describe the kinetics of ethanol fermentation [7], [9]. Equations (1)-(5) below represent the kinetic model:

$$r_x = (r_x)_{growth} + (r_x)_{end} \tag{1}$$

$$\text{where } (r_x)_{growth} = \frac{k_1 XS}{k_2 + S}\exp(-k_5 P) \tag{2}$$

$$r_s = (r_s)_{growth} = -k_3 (r_x)_{growth} \tag{3}$$

$$r_p = (r_p)_{growth} = k_4 (r_x)_{growth} \tag{4}$$

$$(r_x)_{end} = -k_6 X \tag{5}$$

Where $X$, $S$ and $P$ are the biomass, substrate and product concentrations respectively in kg/m$^3$; $r_x$, $r_s$ and $r_p$ are the rates of biomass formation, substrate consumption and product formation.

A set of experimental data of $X$, $S$ and $P$ for different aeration rate (*AR*) and stirrer speed (*SS*) were used to predict the kinetic parameters, $k_1$ to $k_6$. Linear regression model will be used for the identification of the kinetic parameters for different experimental sets under different conditions of *AR* and *SS*. Thus, different kinetic parameters will be obtained for different conditions of *AR* and *SS*. Equation (6) represents the linear regression model used:

$$Variable = \beta_1 + \beta_2 \frac{r - \bar{r}}{\Delta r} + \beta_3 \frac{R - \bar{R}}{\Delta R} \tag{6}$$

Where variable represents predicted $k_1$ to $k_6$, $r$ and R denote *AR* and *SS*, $\bar{r}$ and $\bar{R}$ represent the baseline values for *AR* and SS. $\beta_1$, $\beta_2$ and $\beta_3$ will be obtained through the least squares optimization.

Equations (1)-(6) will be combined and these will form the kinetics model. Clearly in this approach, mixing is integrated by including both *AR* and *SS* in the kinetics model.

*B. Macro-scale Bioreactor Model*

Below shows the macro-scale bioreactor model:

$$\text{Biomass Formation: } dX/dt = r_x \tag{7}$$

$$\text{Substrate Consumption: } dS/dt = r_s \tag{8}$$

$$\text{Product Formation: } dP/dt = r_p \tag{9}$$

Where $X$, $S$ and $P$ are the biomass, substrate and product concentrations respectively in kg/m$^3$; $r_x$, $r_s$ and $r_p$ are the rates of biomass formation, substrate consumption and product formation.

*C. Mixing Model*

The mixing model is developed based on standard k-ε turbulence model and the general reactor model. The k-ε turbulence model is used to describe the mixing mechanism and to govern turbulence in the bioreactor. Equations (10)-(14) below describe the k-ε turbulence model whereas (15) demonstrates the general reactor model:

$$\text{Energy Dissipation: } \varepsilon = (\Delta pFu)/m = (\Delta pu)/(x\rho) \tag{10}$$

Where $\Delta p$ denotes pressure drop, $m$ the mass of the medium, $F$ the cross-section of sampling point and $x$ the axial coordinate.

Fluid flow at constant density fluid:

$$div(\rho u) = 0 \quad \text{(Continuity Equation)} \tag{11}$$

$$div(\rho uk) = div\left(\frac{\mu_{eff}}{\sigma_k} grad\_k\right) + G - \rho\varepsilon \quad \text{(Transport Equation)} \tag{12}$$

$$div(\rho u \varepsilon) = div(\frac{\mu_{eff}}{\sigma_\varepsilon} grad\_\varepsilon) + (C_1 G - C_2 \rho \varepsilon)\frac{\varepsilon}{k}$$

(Transport Equation)(13)

$$u_T = C_\mu \rho \frac{k^2}{\varepsilon} \qquad \text{(Eddy Viscosity)(14)}$$

Where G is the dissipation function $\tau_{ij}\tau_{ij}/(2\mu_{eff})$;

$C_\mu = 0.09; C_1 = 1.44; C_2 = 1.92; \sigma_k = 1.0; \sigma_\Gamma = 1.3$

$$\frac{\delta(\rho\phi)}{\delta t} + \frac{\delta(\rho U_i \phi)}{\delta x_i} = \frac{\delta}{\delta x_i}(\Gamma_\phi \frac{\delta \phi}{\delta x_i}) + S_\phi \qquad (15)$$

Where $\rho$ is the fluid density, $\phi$ 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 a volumetric source term (rate of production of ø per unit volume) of $\phi$. The source term will be equal to the rate based on intrinsic kinetics, i.e. no concentration or temperature gradients within the reactor volume taken under consideration.

All of the equations above, i.e. (1)-(15) will be solved by using CFD software in order to predict the yield and productivity of the fermentation process under different *AR* and *SS*. Predicted yield and productivity will be compared with experimental yield and productivity in order to determine the accuracy of the proposed multi-scale model.

Equations (16)-(17) represent the yield and productivity determination:

$$Yield = \frac{P}{S_0 - S} \times 100\% \qquad (16)$$

$$\text{Pr}oductivity = \frac{P}{BT} \qquad (17)$$

Where $S_0$ is the initial substrate concentration (kg/m$^3$) and *BT* is the batch time (s) allocated for the fermentation process.

## III. EXPERIMENTAL SET-UP

The bioreactor used is the BIOSTAT A Plus, MO-Assembly as shown in Fig. 2. Saccharomyces cerevisiae (Baker's Yeast) is utilized as the inoculum culture with glucose as the main substrate. Approximately 0.001g of Baker's Yeast is added into the inoculum for microbial growth. The inoculum is allowed to stand for 28,800s (8 hours) under room temperature to be cultured. 0.0015m$^3$ (1.5L) of fermentation medium is prepared by adding 0.075kg glucose, 0.0075kg yeast, 0.00375kg NH4Cl, 0.00437kg Na2HPO4, 0.0045kg $KH2PO_4$, 0.00038kg $MgSO_4$, 0.00012kg $CaCl_2$, 0.00645kg citric acid and 0.0045kg sodium citrate. The medium culture is sterilized at 121$^o$C for 900s (15 minutes) and cooled down under room temperature. 4x10-5m3 (0.040L) of inoculum is added to the fermentation medium. Temperature and pH conditions are maintained and controlled at 30°C and pH 5 respectively. Three different sets of experiment will be conducted based on different AR and *SS* within certain range of AR and *SS*, i.e. 1.67x10$^{-5}$-2.505x10$^{-5}$m$^3$/s (1.0-1.5LPM) AR and 2.500-4.167rps (150-250rpm) SS. Air is supplied from the sparger which is located at the bottom of the bioreactor. The batch process is stopped after approximately 259,200s (72 hours) and samples are taken in every 7,200s (2 hours) to be analyzed for ethanol, glucose and biomass concentrations by using R-Biopharm test kits and Lambda Perkin, UV-spectrophotometer. Same experimental set-up will be conducted for different experiments under different *AR* and *SS*.

Figure 2. BIOSTAT A Plus, MO-Assembly Bioreactor.

## IV. RESULTS AND DISCUSSION

In this section, experimental and CFD results are presented based on yield and productivity data obtained. Both results are compared to ensure that the kinetics multi-scale model embedded is suitable to describe the non-ideally mixed behavior of the bioreactor.

### *A. Yield*

Table I shows the summary results for both experimental and simulated yield for different conditions of *AR* and *SS*. Based on Table I, the experimental and simulated yield for all experiments are quite comparable, whereby the lowest difference is observed under experiment 2 with 0.92% difference. On the other hand, the highest difference is 5.00% under experiment 1. These data showed that the kinetics multi-scale model is suitable in predicting the yield of the fermentation process within the experimental range. Thus, the kinetics multi-scale model is suitable in describing the mixing behavior in terms of yield process within 5.00% error.

Based on the results, it would be interesting to look at the

mixing behavior through CFD profile. Figs. 3-5 represent the CFD mixing profile in terms of yield for each experiment. Samples were taken at the sampling point as shown in each figure, whereby the sampling point is similar for each experiment for consistency. Each profile demonstrated different profiles, especially for experiment 1. As shown in Fig. 3 below, the yield is concentrated around the stirrer blades and at the bottom of the bioreactor vessel. These show that mixing is concentrated around the stirrer blades and beneath the bioreactor vessel, thus stirrer speed and aeration rate play an important role in the mixing mechanism.

TABLE I. SUMMARY OF EXPERIMENTAL AND SIMULATED YIELD

| Exp | *AR* ($m^3/s$) | *SS* (rps) | Exp. Yield (%) | Simulated Yield (%) | % Diff. |
|---|---|---|---|---|---|
| 1 | $1.67x10^{-5}$ | 4.17 | 15.105 | 15.900 | 5.00 |
| 2 | $2.08x10^{-5}$ | 3.33 | 21.500 | 21.700 | 0.92 |
| 3 | $2.50x10^{-5}$ | 2.50 | 16.392 | 17.000 | 3.58 |

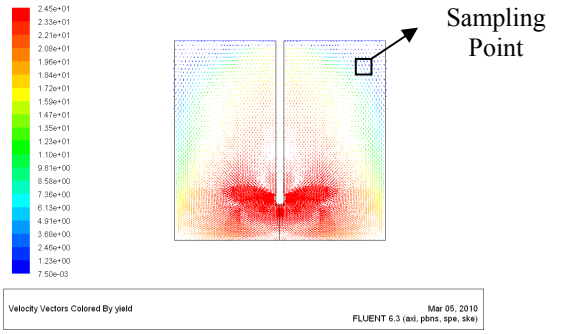

Figure 3. Velocity Vector of Yield (1.67x10-5m3/s AR, 4.17 SS).

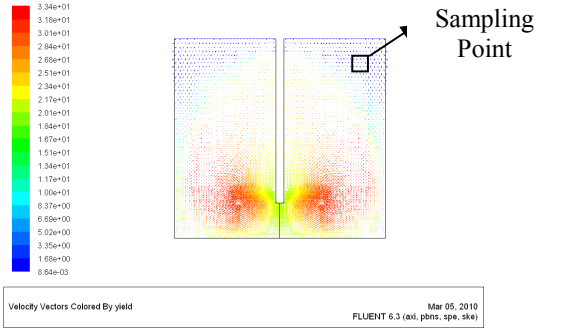

Figure 4. Velocity Vector of Yield (2.08x10-5m3/s AR, 3.33 SS).

Compared to experiments 2 and 3, the profiles are slightly different, whereby yield is concentrated by the sides of the impeller blades too but not around the bottom of the bioreactor vessel. From Fig. 4 and Fig. 5, the profiles are comparably similar. The difference is that yield is more concentrated around the stirrer blades for experiment 2 as compared to experiment 3. This showed that at lower *AR* and higher *SS*, yield is more concentrated around the impeller blades and towards the bottom of the bioreactor vessel, but with lower value of yield as showed in Fig. 4 and Fig. 5, the highest yield attained for experiment 2 is 33.40% and 37.70% for experiment 3. Overall, it is suggested that, the highest percentage yield could be obtained at $2.08x10^{-5}m^3/s$ and 3.33rps, which is the baseline of the experimental range. The difference between experimental and simulated yield for this condition is also the lowest, thus it is suitable to predict yield under this condition.

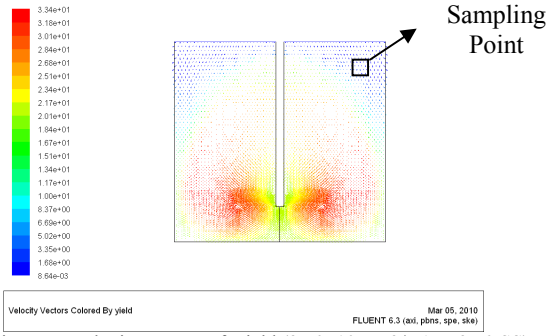

Figure 5. Velocity Vector of Yield (2.50x10-5m3/s AR, 2.50 SS).

## B. *Productivity*

Table II shows the summary results for both experimental and simulated productivity for different conditions of *AR* and *SS*. Experimental setups are similar as in Table I.

TABLE II. SUMMARY OF EXPERIMENTAL AND SIMULATED PRODUCTIVITY

| Exp | *AR* ($m^3/s$) | *SS* (rps) | Exp. Prod. ($kg/m^3.s$) | Simulated Prod. ($kg/m^3.s$) | % Diff. |
|---|---|---|---|---|---|
| 1 | $1.67x10^{-5}$ | 4.17 | $2.83x10^{-5}$ | $3.28x10^{-5}$ | 13.72 |
| 2 | $2.08x10^{-5}$ | 3.33 | $5.00x10^{-5}$ | $5.63x10^{-5}$ | 11.19 |
| 3 | $2.50x10^{-5}$ | 2.50 | $2.94x10^{-5}$ | $3.06x10^{-5}$ | 3.92 |

Experimental and simulated productivity are quite comparable for all experiments with differences from 3.92% to 13.72%. These results showed that the kinetics multi-scale model could predict the productivity of the fermentation process approximately within 14.00% error. Interestingly, the highest difference, i.e. 13.72% is observed from experiment 1. Compared to yield prediction, the highest difference of 5.00% is observed. This showed that the kinetics multi-scale model could predict yield better than productivity within experimental range. Productivity predictions based on the kinetics multi-scale model is more accurate for higher *AR* and lower *SS*, based on the differences tabulated in Table II.

Similar as the CFD profiles for yield, Figs. 6-8 represent the CFD mixing profiles in terms of productivity for each experiment. The CFD profile for experiment 1 as shown in Fig. 6 below demonstrated that the highest productivity value is $6.56x10^{-5}$m3/kg.s, which is concentrated around the stirrer blades and at the bottom of the bioreactor vessel. The profile for productivity is similar as the profile for yield. This showed that both aeration rate and stirrer speed are vital parameters in the determination of yield and productivity in order to investigate the mixing behavior of the bioreactor. On the other hand, Fig. 7 and Fig. 8 represent the CFD profiles for experiment 2 and experiment 3. The profiles are slightly different compared to experiment 1 as productivity is concentrated by the sides of the stirrer blades and not at the bottom of the bioreactor vessel. Based on the profiles, the highest productivity simulated from experiment

2 is 8.67x10-5 m3/kg.s whereas for experiment 3, the highest productivity simulated is 7.62x10-5m3/kg.s.

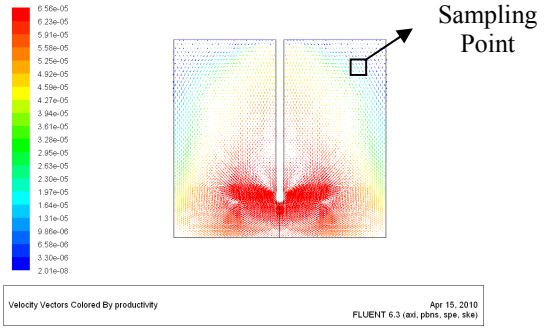


Figure 6. Velocity Vector of Productivity (1.67x10-5m3/s AR, 4.17 SS).

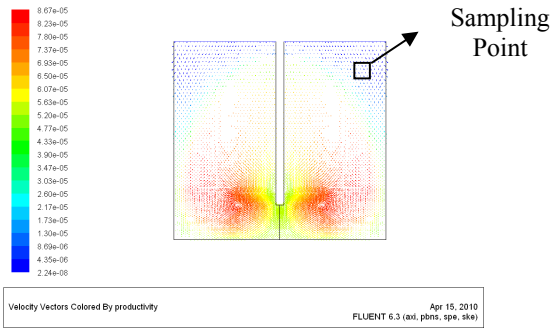


Figure 7. Velocity Vector of Productivity (2.08x10-5m3/s AR, 3.33 SS).

Based on Fig. 7, productivity is concentrated more by the side of the stirrer blades and towards to the bottom of the bioreactor vessel. On the other hand, for experiment 3, based on Fig. 8, productivity is concentrated by the side of the stirrer blades but not towards to the bottom of the bioreactor vessel. These results showed that productivity predictions based on the kinetics multi-scale model is more accurate at higher *AR* and lower SS. Thus, the profile for Fig. 8 is suggested to be more precise in investigating the mixing behavior within the bioreactor vessel. Overall, based on all CFD profiles for yield and productivity, it is suggested that with the implementation of the kinetics multi-scale model, the baseline values for AR and SS is more accurate in yield prediction whereas for productivity prediction, it is more accurate at higher AR and lower SS. It would be interesting to widen the experimental range of both AR and SS in order to investigate whether the proposed kinetics multi-scale model is suitable to predict other conditions of AR and SS. This would be useful for predictions for a wide range of AR and SS, without actually undergoing experiments which is costly and time consuming. On the other hand, since different conditions of AR and SS will give different predictions for yield and productivity through CFD, it would be interesting to find the optimized conditions of AR and *SS* to observe whether the optimized conditions will obtain higher predictions of yield and productivity at the same time. This will ensure that both yield and productivity are maximized at the same time rather than conducting 2 different analysis for 2 different operating conditions to obtain maximum yield and productivity. This will save time and cost in terms of experimental and computational burden.

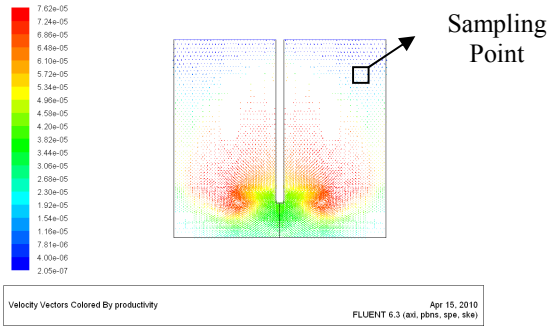


Figure 8. Velocity Vector of Productivity (2.50x10-5m3/s AR, 2.50 SS).

## V. CONCLUSION

Most of the models proposed so far, deviate from ideal mixing behavior, without considering the mixing mechanism within the bioreactor. This could lead to severe loss in yield and changes in microbial physiology. Thus, a kinetics multi-scale model is proposed in order to describe the non-ideally mixing mechanism of the bioreactor. Aeration rate and stirrer speed are implemented into the proposed model to study the effect of both parameters in the mixing mechanism of the bioreactor. Comparisons between experimental and CFD simulations have been done to investigate whether the proposed kinetics multi-scale model is suitable and precise to be utilized under certain range of aeration rate and stirrer speed. Results suggested that yield predictions from CFD simulations gave rise to approximately 5.00% error compared to yield results obtained from experiment. On the other hand, around 14.00% error is observed for the predictions of productivity. Thus, these suggest that the kinetics multi-scale model is suitable and precise to be utilized for yield and productivity predictions within certain range of aeration rate and stirrer speed. With the implementation of the proposed kinetics multi-scale model into CFD simulations, the non-ideally mixed mechanism of the bioreactor could be observed and could enhance the physiology of the fermentation process.

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