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# Mass Transfer Effects and Kinetics of Gaden Type I Fermentations Using Immobilized Whole-Cells in a CSTB

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An unstructured model for growth associated Gaden type I fermentations for continuously stirred tank bioreactor (CSTB) with immobilized whole-cells is presented. Based on the developed model equation an experimental method has been evolved for the determination of kinetic parameters and effectiveness factor. Intraparticle diffusion limitations and inactivation due to immobilization process have been incorporated using effectiveness factor concept. The method has been illustrated by ethanol fermentation using alginate entrapped *Sacharomvces cerevisiae* cells. The maximum productivity of the CSTB has also been determined. The model predicted $D_{\max}$ values are found to be in good agreement with the experimental values.

## 1. Introduction

There is an increasing interest in the practical applications of immobilized microbial cell systems for the production of biochemicals. The emphasis on enzyme immobilization during the last two and half decades has resulted in the development of new immobilization techniques, many of which, are equally applicable to cells. This has provided an impetus for re-examining the conventional processes employing free cells vis-a-vis immobilized whole cells with a view to improve the reactor productivity. A large number of immobilization techniques are now available as given by Karel et al. (1985). The immobilization of whole cells also affects the catalytic activity of the cells. Considerable efforts have been made by researchers to employ theory of reaction and diffusion in porous media to immobilized enzymes and whole cell systems (Andrews, (1988); Nakasaki, (1989); Hannoun and Stephanopoulous, (1990); Teixeira and Mota (1990); Mitchellet et al. (2004), Tatjana et al. (2006)). In the present paper, the approach given by Prasad and Mishra (1995) has been extended to a continuously stirred tank bioreactor and unstructured model for the Gaden type I fermentations presented. A method has also been developed based on the model equation for the determination of kinetic parameters and the effectiveness factor from the CSTB data.

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Ethanol fermentation data of CSTB are used for the illustration of the method. The experimental and theoretical dilution rates corresponding to maximum reactor productivity rates are also discussed.

## 2. Development

As detailed by Prasad and Mishra (1995), using inhibition-free Monod kinetic expression for cell growth and the relation for substrate consumption, cell growth and product formation rate via corresponding yield factors and incorporating the free cell contribution in the overall reaction for the growing immobilized cell system, the rate of substrate consumption is given by:

$$-r_s = \frac{\mu_{\max}}{Y_{x/s}} X \frac{S}{S+K_m}\varepsilon_1 + \eta \frac{\mu_{\max}}{Y_{x/s}} MS' X_s' \frac{S}{S+K_m}\varepsilon_s \tag{1}$$

Here the steady sate operation of the continuously stirred tank bioreactor has been assumed where immobilization matrix is loaded to its maximum retaining capability and the growth of biomass on immobilization matrix is such that the new cells formed get leached instantaneously into free space and equilibrium is maintained all through as detailed by Prasad (1991) and Prasad and Mishra (1995). The gas hold-up has been considered to be negligible as also used by Okita and Kirwan (1986). With the above assumptions, the steady state material balance around the CSTB for the substrate may be written as

$$F\left(S_o - S\right) = (-r_s)V \tag{2}$$

where $(-r_s)$ is substrate utilization rate in the reactor as defined by eqn (1).

Defining the dilution rate ( $D$ ) on the basis of actual liquid hold-up $(D = F/V\varepsilon_1)$ and substituting $(-r_s)$ from eqn (1) in eqn (2) and then simplifying, one gets

$$D(S_o - S) = \frac{\mu_{\max}}{Y_{x/s}}\left[X + \eta MS' X_s'\right]\frac{S}{S+K_m} \tag{3}$$

Where $MSX_s'$ is the steady state immobilized cell concentration in the reactor based on actual liquid volume in reactor and is defined as

$$MSX_s' = \frac{MS' X_s' \varepsilon_s}{\varepsilon_1} \tag{4}$$

The biomass yield $Y_{x/s}$ may be defined as

$$Y_{x/s} = \frac{X - X_o}{S_o - S} \tag{5}$$

and could be used for relating exit free cell and substrate concentrations for Gaden type I fermentations. On combining eqn (3) and eqn (5), one gets the following equation which describes the performance of CSTB using immobilized whole cells:

$$D(S_o - S) = \frac{\mu_{\max}}{Y_{x/s}}[X_o + Y_{x/s}(S_o - S) + \eta MSX_s']\frac{S}{S + K_m} \tag{6}$$

Substitution of $MSX_s' = 0$ in eqn (6) results in the equation for Monod Chemostat as given by Bailey and Ollis (1977).

## 3. Maximum Productivity

As detailed by Bailey and Ollis (1977), the maximum productivity of free cells or product for Gaden type I fermentations is obtained by maximizing the cell production rate $(DX)$ in the reactor. Therefore the condition for maximum cell or product formation rate may be obtained by differentiating the cell production rate $(DX)$ with respect to dilution rate ( $D$ ) and equating it to zero. Replacing $D$ by $D_{\max}$, we get

$$\mu_{\max}[2D_{\max}Y_{x/s}/(S_o + K_m) - \mu_{\max}S_oY_{x/s} + \mu_{\max}\eta MSX_s' - D_{\max}{}^2Y_{x/s}(S_o + K_m)$$
$$+[[D_{\max}Y_{x/s}/(S_o + K_m) - \mu_{\max}S_oY_{x/s} + \mu_{\max}\eta MSX_s']^2 + 4(\mu_{\max} - D_{\max})\mu_{\max}\eta MSX_s'S_oY_{x/s}]^{1/2}$$
$$*\left[\mu_{\max} + \frac{D_{\max}(\mu_{\max} - D_{\max})BY_{x/s}(S_o + K_m) - 2\mu_{\max}\eta MSX_s'S_oY_{x/s}}{B^2 + 4(\mu_{\max} - D_{\max})\mu_{\max}\eta MSX_s'S_oY_{x/s}}\right] \tag{7}$$

where $B = D_{\max}Y_{x/s}(S_o + K_m) - \mu_{\max}S_o + \mu_{\max} + \eta MSX_s']$

Eqn (7) cannot be solved explicitly for $D_{\max}$ Hence a trial and error procedure has to be employed for the determination of $D_{\max}$. The substitution of $MSX_s' = 0$ in eqn (7). results in the equation for $D_{\max}$, for free cells in a CSTR with sterile feed as has been given by Bailey and Ollis (1977).

## 4. Method For The Determination Of Kinetic Parameters And The Effectiveness Factor

The biomass yield $(Y_{x/s})$ can be determined under steady-state conditions by measuring free cell and substrate concentrations at different dilution rates and then averaged over the range of dilution rates. Maximum specific growth rate $(\mu_{\max})$, Monod constant $(K_m)$ and the effectiveness factor (η) can be determined by using the following procedure:

Rearranging eqn (6), one may get

$$\frac{X_o + Y_{x/s}(S_o - S) + \eta MSX_s'}{D(S_o - S)Y_{x/s}} = \frac{K_m}{\mu_{\max}}\frac{1}{S} + \frac{1}{\mu_{\max}}$$

Thus $[X_o + Y_{x/s}(S_o - S) + \eta MSX_s']/[D(S_o - S)Y_{x/s}]$ may be plotted against (1/S) giving a slope of $(K_m/\mu_{\max})$ and an intercept of $(1/\mu_{\max})$.For a set of experimental

values of dilution rate and exit substrate concentration and with the experimentally known values of $X_o$, $MSXs'$ and $Y_{x/s}$, the ordinate of the plot cannot be determined due to unknown value of $\eta$. Therefore, a trial and error procedure using different values of $\eta$ and then fitting the equation with the experimental data points and determining correlation coefficient for each line corresponding to the used value of η is followed. That value of $\eta$ which gives maximum value of correlation coefficient (nearest to 1.0) is then selected and the best fit straight line is drawn through the data points plotted as $\frac{X_o + Y_{x/s}(S_o - S) + \eta MSX_s'}{D(S_o - S)Y_{x/s}}$ against (1/S). The slope and intercept from this plot are then used for the determination of $\mu_{max}$ and $K_{max}$ ).

## 5. Methods

Ethanol fermentation by *Saccharomyces cerevisiae* cells immobilized in calcium alginate matrix was selected due to its simplicity and mild conditions of immobilization as an example of Gaden type I fermentations. The CSTB experiments were carried out in a Biostat M fermentor of 2.01 capacity. Details of cell cultivation, immobilization of cells, the determination of the physical properties of the immobilized cells and matrix, analysis of substrate and product, etc. are provided by Prasad (1991) and Prasad and Mishra (1995).

## 6. Results And Discussion

### 6.1 Illustration of the method for the determination of kinetic parameters and effectiveness factor

The exit substrate concentration profile against the dilution rate as shown in Fig 1 for a particular run for the sterile feed has been used in the illustration. The biomass yield ( $Y_{x/s}$ ) was determined under steady-state conditions at each dilution rate by measuring the free cell concentration and glucose concentration in the exit stream of CSTB. The arithmetic average value of $Y_{x/s}$ over these dilution rates was found to be 0.0887 kg/kg and has been used subsequently in the calculations.

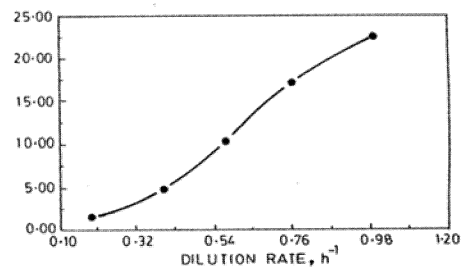


*Fig. 1 Exit substrate concentration profile*

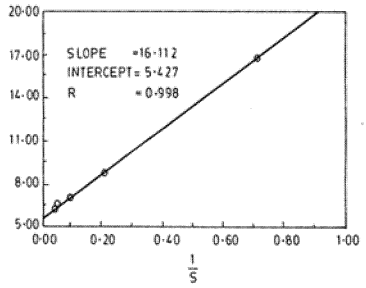


*Fig. 2 Plot of* $\frac{X_o + Y_{x/s}(S_o - S) + \eta MSX_s'}{D(S_o - S)Y_{x/s}}$ *against 1/S*

The value of effectiveness factor, $\eta$ was determined from the linear regression analysis of the experimental data giving highest value of correlation coefficient. For the present set of exit substrate concentrations and dilution rates $\eta$ was found to be 0.317. The correlation coefficient for this value of $\eta$ was found to be maximum (0.973). From a plot of $[X_o + Y_{x/s}(S_o - S) + \eta MSX_s']/[D(S_o - S)Y_{x/s}]$ against (1/S) for $\eta$ = 0.317 as shown in Fig. 2, The slope and intercept were determined as 16.112 and 5.427, respectively. The values of $\mu_{max}$ and $K_m$ were then found as $\mu_{max}$ = 0.184 $h^{-1}$ and $K_m$ = 2.296 $kg/m^3$

**6.2 Maximum productivity**

Under steady state growth conditions, the effect of inlet substrate concentration on the productivity of ethanol, exit substrate and free cell concentrations was investigated. Four inlet substrate concentrations, namely, 25, 50, 75 and 100 $kg/m^3$ were used in these experiments. The variation of substrate (glucose), product (ethanol), and free cell concentrations in the reactor and ethanol productivity with dilution is given elsewhere (Prasad 1991). The ethanol productivity shows an increasing trend with the dilution rate reaches the peak value and then maintains a decreasing trend. Using eqn (7) $D_{max}$ values for different inlet substrate concentrations were determined by trial and error procedure and these are given in Table 1. Maximum ethanol productivities as determined experimentally are also given in this table.

*Table 1 $D_{max}$ and $PD_{max}$ for different inlet substrate concentrations*

| Inlet substrate concentration, $S_o$, $kg/m^3$ | 25 | 50 | 75 | 100 |
|---|---|---|---|---|
| Dilution rate for maximum productivity, $D_{max}$, $h^{-1}$ | | | | |
| i. Experimental | 0.805 | 708 | 0.521 | 0.512 |
| ii. Model predicted | 0.859 | 0.663 | 0.557 | 0.473 |
| iii. % deviation | +6.72 | +6.36 | +6.93 | -7.63 |
| Maximume ethanol productivity | | | | |
| Experimental | | | | |
| $PD_{max}$ ($kg/m^3$.h) | 4.68 | 9.75 | 13.85 | 16.05 |

It may be seen from this table that the experimental results are well represented by the present model; the maximum deviation of model predicted values from the experimental data being not more than ±7.6%. The dilution rate corresponding to maximum productivity decreases while the maximum productivity of the CSTB increases as the inlet substrate concentration increases from 25 to 100 $kg/m^3$.

## 7. Conclusions

A new experimental method has been evolved for the determination of effectiveness factor of the immobilized whole cells from continuously stirred tank bioreactor. The strain specific kinetic parameters are also obtained from this method. The dilution rate corresponding to maximum reactor productivity can also be determined from the proposed model equation.

## Nomenclature

| | |
|---|---|
| D | Dilution rate $(F/V\varepsilon_l)$, $h^{-1}$ |
| $D_{max}$ | Dilution rate corresponding to maximum productivity, $h^{-1}$ |
| F | Flow rate, $m^3/h$ |
| $K_m$ | Monod constant for growth, $kg/m^3$ |
| $MS'$ | Support concentration of immobilized matrix, $kg/m^3$ |
| $MS'X'$ | Concentration of immobilized cells in the immobilization matrix ,$kg/m^3$ |
| $MS$ | Support concentration in reactor $(MS'\varepsilon_s/\varepsilon_l)$, $kg/m^3$ of liquid hold up |
| $MSX'$ | Concentration of immobilized cells per unit liquid volume in reactor, $kg/m^3$ |
| P | Product (ethanol) concentration, $kg/m^3$ |
| $r_s$ | Rate of substrate utilization in reactor, $kg/(m^3.h)$ |
| S | Substrate concentration, $kg/m^3$ |
| t | Time, h |
| V | Volume of the reactor,$m^3$ |
| X | Free cell concentration, $kg/m^3$ |
| $X'$ | Immobilized cell loading, kg/kg |
| $Y_{x/s}$ | Yield of biomass based on total substrate in reactor, kg/kg |
| $\varepsilon_l$ | Liquid hold up in reactor, dimensionless |
| $\varepsilon_s$ | Immobilization matrix (gel bands) hold up in reactor, dimensionless |
| $\mu_{max}$ | Maximum specific growth rate $h^{-1}$ |
| $\eta$ | Effectiveness factor, dimensionless |

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