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A Distributed Parameter Diffusion-Reaction Model for the Alcoholic Fermentation Process

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ABSTRACT

A distributed parameter model is developed for the yeast floc in the alcoholic fermentation process. The model takes into consideration the external mass transfer resistances, the mass transfer resistance through the cellular membrane, and the diffusion resistances inside the floc. The two-point boundary value differential equations for the membrane are manipulated analytically, whereas the nonlinear two-point boundary value differential equations of diffusion and reaction inside the floc have been approximated using the orthogonal collocation technique.

The evaluation of the necessary diffusion coefficients have involved a relatively large number of assumptions because of the present limited knowledge regarding the complex process of diffusion and biochemical reactions in these systems.

The model results have been compared with the experimental results of a laboratory batch fermentor and the results of an industrial fed-batch fermentor. The model simulates reasonably well the experimental results, with the largest deviation being for the concentration of yeast.

Index Entries: Alcoholic fermentation; ethanol; diffusion-reaction; mathematical modeling; simulation of fermentors; yeast; *S. cerevisiae;* flocculation.

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NOMENCLATURE

C_1, C_2, C_1', C_2'	integration constants
Di_k	Diffusion coefficient of component i in phase k, cm^2/sec
K_1, K_2	Equilibrium partition constant for the floc side of the membrane
Kg_i	External mass transfer coefficient for component i, cm/sec
K_s	Saturation constant, g/cm^3
K_p	Inhibition constant, g/cm^3
K_p'	Rate constant, g/cm^3
N_i	Flux of component i, $g/cm^2 \cdot sec$
N	Toxic factor
p	Intracellular ethanol concentration, g/cm^3
p_c	Ethanol concentration at the internal collocation point, g/cm^3
p_b	Extracellular ethanol concentration, g/cm^3
r	Radial coordinate from the center of the floc, cm
R_1	Internal radius of membrane, cm
R_2	External radius of membrane, cm
S	Intracellular sugar concentration, g/cm^3
S_c	Sugar concentration at the internal collocation point, g/cm^3
S_b	Extracellular sugar concentration, g/cm^3
t	Time, seconds
x_i	Mole fraction of component i
X	Biomass concentration, g dry wt/cm^3
X_m	Maximum biomass concentration, g dry wt/cm^3
Y_c	Yield factor for yeast, g yeast produced/g sugar consumed
Y_p	Yield factor for ethanol, g ethanol produced/g sugar consumed

GREEK SYMBOLS

α_{22}	Constant related to Eqs. (2) and (A-11)
μ_i	Viscosity of component i, C.P.
μ_m	Maximum specific growth rate, sec^{-1}
ρ_d	Dry density of floc, g dry wt/cm^3 wet vol ($=0.2$ g/cm^3)
ρ_ω	Density of floc, g wet wt/cm^3 wet vol ($=1.23$ g/cm^3)
ω	r/R_1
ω_2	R_2/R_1
ω_1	$R_1/R_1=1.0$
ω_1^+	$R_1^+/R_1=1.0^+$
ω_1^-	$R_1^-/R_1=1.0^-$

INTRODUCTION

In a previous paper (*1*), a heterogeneous model for the alcoholic fermentation process was developed. The model takes into consideration the diffusional resistances between the bulk of the fluid and intracellular fluid for both sugar and ethanol. For that model the diffusional resistance for the ethanol was obtained empirically by fitting the model to one set of experimental results for intracellular and extracellular ethanol concentrations reported by Novak et al. (*2*) during batch fermentation. The mass transfer coefficient for ethanol diffusion between the intracellular and extracellular fluids was found to vary with the degree of conversion (*1*).

The model was checked successfully against another set of experimental results for batch fermentation. Furthermore the model was modified to represent the fed-batch fermentation and was used to simulate an industrial fed-batch fermentor. The agreement between the model and the industrial fermentor was quite good (*1,3*).

In this paper, the heterogeneous model developed earlier (*1*) is being developed to get rid of the empirical nature of the ethanol mass transfer coefficient, and also to get a better representation of the intracellular-extracellular diffusion of sugar. This is carried out at this preliminary stage of model development by taking three mass transfer resistances in series for both ethanol and sugar, these are:

1. External mass transfer resistances that depend on the physical properties of the bulk fluid (extracellular fluid) and the degree of mixing as well as temperature.
2. Diffusion through the cell membrane: a process that is not fully understood and which is affected by many factors in a complex way (e.g., it is well known that ethanol increases the fluidity of biological membranes (*4*), besides, ethanol causes a change in the phospholipid composition and a decrease of the lipid to protein ratio of the membrane (*5*)). At steady state the transport of sugar through biological membranes is considered a carrier-mediated process, which consists of diffusion and biochemical reaction in the membrane (*6,49*). Glucose diffusion against concentration gradient has been beautifully simulated by Kernevez for the glucose pump formed of artificial membranes (*7*). However, for the present unsteady state model all bioreactions are assumed to take place inside the cell with the assumption that no reaction is taking place in the membrane. Therefore Fickian diffusion of sugar is considered through the membrane with the appropriate diffusion coefficient.
3. Intracellular diffusion, which is dependent on the intracellular conditions. A reasonably rigorous description of this diffusion process requires a distributed diffusion-reaction model. A more rigorous description, which is not considered in this paper, requires a structured diffusion-reaction model.

Background on the Problems Associated with the Heterogeneous Modelling of Alcoholic Fermentation Processes

Rigorous heterogeneous modeling of alcoholic fermentation process requires a large amount of fundamental information which is not completely available in the literature now. However, rational mathematical models based on the available knowledge regarding this process represent considerable improvement over the usual pseudo-homogeneous models as well as helping to direct experimental investigations in a more organized manner.

The alcoholic fermentation process is quite a complex process and involves the following basic processes.

Product Inhibition

The ethanol inhibition effect is of the noncompetitive type, where ethanol concentration affects only the maximum specific growth rate. This dependence of the maximum specific rate of growth on ethanol concentration involves two main types of dependence; linear dependence (*8,9*) and nonlinear dependence (*10–12*). Jobses and Roels (*13*) have shown that inhibition kinetics can be approximated by a linear relation between the specific growth rate and the ethanol concentration up to 50 g/L ethanol. Above this level, deviation from linearity is observed.

As discussed in ref. (*1*), many investigators reported that produced ethanol (intracellular ethanol) is more toxic than added ethanol (extracellular ethanol) (*2,14–19*), and an intermediate value of $K_p = 35$ has been obtained, which lies between $K_p = 105.2$ g/L for added ethanol and $K_p = 3.04$ g/L for produced ethanol. This value is believed to represent K_p for ethanol inhibition when using the heterogeneous model, which distinguishes between intracellular and extracellular fluids.

Sugar Inhibition

It is well known that high concentration of sugar inhibits the growth of yeast (*20–23*). However, the inhibitory effect of sugar is negligible below sugar concentration of 100 g/L (*24*). The inhibitory effect of sugar is not included in the present model, however it can easily be added to the kinetic rate equation without any extra complications.

Cell Inhibition

In alcoholic fermentation most kinetic models express cell growth rate as a linear function of cell concentration. However, the experimental results of Cysewski and Wilke (*25*) and Lee et al. (*26*) showed that at high cell concentration the linear relationship is incorrect.

Ethanol Diffusion

The dispute in the literature regarding the diffusion of ethanol (*14–19, 27–29*) has been discussed in ref. (*1*). The slightly more detailed diffusion

reaction model for the alcoholic fermentation process presented in this paper will help to throw more light on this disupte than the model used in ref. (*1*). However, we are certainly still quite far from a completely rigorous representation of this rather complex diffusion-reaction problem.

Flocculation of the Yeast

The flocculation of the yeast affects the mass transfer rates and thus the rate of fermentation considerably through the change of the exposed surface area for mass transfer per unit mass of the microorganism. The flocculation process has been investigated extensively experimentally (*30–38*). However, the theoretical basis for understanding the flocculation process is severely lacking.

Development of the Model

The model equations are developed for the flocs with three mass transfer resistances in series for sugar and ethanol. The model is quite different from the floc model of ref. (*1*), whereas for the extracellular fluid the model does not differ from that of ref. (*1*).

Single Floc Model

The floc is formed of a number of cells. This number depends on many factors including the liquid phase composition and the flocculating tendency of the specific strain of yeast. The structure of the floc is quite complex and branched, giving rise to what may be called a fractal structure (*40*). Davis and Hunt (*41*), in their investigation of the separation of yeast flocs by settling, used the geometric diameter of the fractal structure to compute the settling velocity of the yeast. In the present work we use the more classical approach of reducing the complex structure of the floc to an equivalent sphere. The geometric dimensions of the floc fractal structure can be used instead. However, there are certain difficulties associated with the determination of the fractal dimension f, and the constant of proportionality α (needed for the calculation of the geometric dimension of the fractal structure, $D_s=(N/\alpha)^{1/f}$, where N is the number of cells forming the floc), which are beyond the scope of the present paper.

Fig. 1 shows the idealized equivalent sphere used in this model, where the internal of floc (the equivalent sphere) is considered a substrate sink where sugar reacts to produce ethanol.

Diffusion through the cell membrane (the membrane of the floc equivalent sphere) is assumed to obey Fick's law with membrane diffusion coefficients that take into account the complex nature of the membrane. Carrier mediated transport of sugar through the membrane is not considered because of the unsteady state nature of the model and the fact that the model assumes all the bioreactions to take place inside the cell with no reactions taking place in the membrane. The external mass transfer re-

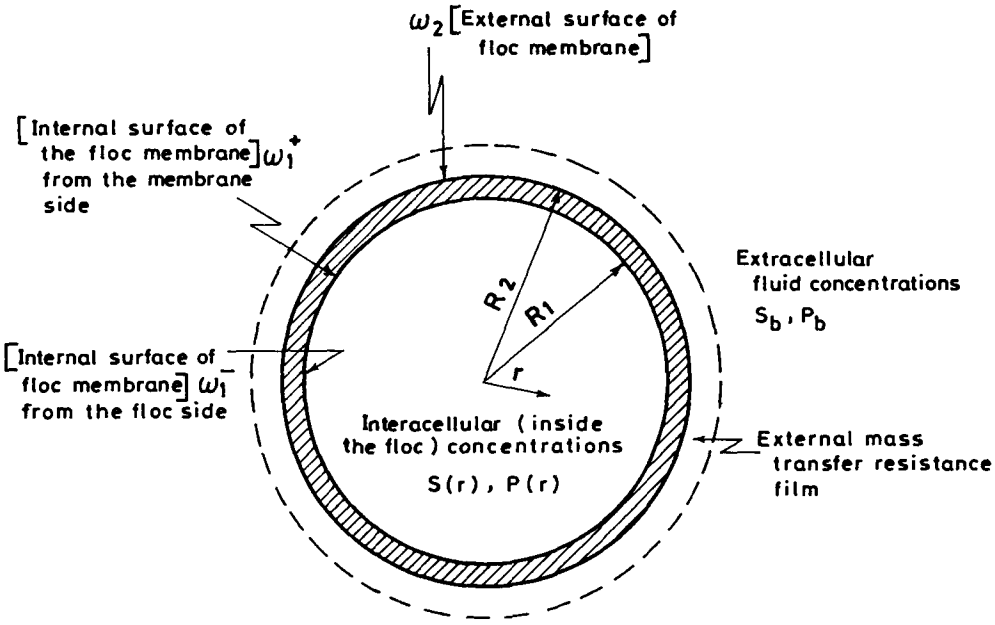

Fig. 1. Schematic presentation of the proposed model of an equivalent sphere for the microbial floc.

sistances are assumed to be lumped in one hypothetical external mass transfer film, with the mass transfer coefficient depending on the extracellular conditions.

Single Floc Equations

A schematic diagram showing the details of the proposed model for the equivalent sphere representing the microbial floc is shown in Fig. 1.

Mass Balance of Ethanol Within the Membrane

If we assume no reaction is taking place in the membrane, then the diffusion equation becomes:

$$dN_p / d\omega = d/d\omega[-Dp_m \cdot \omega^2 \cdot d_p/d\omega] = 0 \quad (1)$$

where,

$$\omega = r/R_1$$

The diffusivity of ethanol through the membrane is estimated as a function of ethanol mole fraction in the membrane x_1 by the following relation (see Appendix 5):

$$Dp_m = \alpha_{22} / [x_1 \mu_1^{1/3} + (1-x_1) \mu_2^{1/3}]^3 \quad (2)$$

where,

$$x_1 = p / [\rho\omega \cdot R_M + p(1-R_M)] \tag{3}$$

where R_M is the ratio between the mol wt for ethanol and the membrane lipid.

Substitution of Eqs. (2) and (3) into Eq. (1) and integration gives:

$$f(p) = \alpha_{22}[A \cdot p + B/d \ln(c+d \cdot p) - A_1 / d(c+d \cdot p) - A_2 / 2d(c+d \cdot p)^2] = C_1 - C_2/\omega \tag{4}$$

where C_1 and C_2 are the constants of integration to be fitted to the boundary conditions.

The physical constants A, B, C, d are given in Appendix (9).

Boundary Condition at the External Surface of the Membrane ($r = R_2$, i.e., $\omega = R_2/R_1$)

The boundary condition at this point is given by:

$$dp / d\omega \,|_{\omega 2} = (K_{gp} \cdot R_1 / Dp_{m(\omega 2)})(p_b - p_{\omega 2}) \tag{5}$$

From Eq. (1) we obtain:

$$dp / d\omega \,|_{\omega 2} = C_2 / (Dp_{m(\omega 2)} \omega_2^2) \tag{6}$$

Thus, from Eqs. (5) and (6), we can express the constant of integration C_2 in terms of the concentration at the point ω_2 ($=R_2/R_1$):

$$C_2 = (R_2^2/R_1) Kg_p (p_b - p_{\omega 2}) \tag{7}$$

Equation (1) at the point ω_2 can be written as:

$$f(p_{\omega 2}) = \alpha_{22}[A \cdot p_{\omega 2} + (B/d) \ln(c+d \cdot p_{\omega 2}) - \{A_1/d(c+d \cdot p_{\omega 2})\} - \{A_2/2d(c+d \cdot p_{\omega 2})^2\}] = C_1 - C_2/\omega_2 = C_1 - R_2 \cdot Kg_p (p_b - p_{\omega 2}) \tag{8}$$

Therefore C_1 can also be written in terms of $p_{\omega 2}$ as follows:

$$C_1 = f(p_{\omega 2}) + R_2 \cdot Kg_p (p_b - p_{\omega 2}) \tag{9}$$

At the point ω_1^+ ($R_1^+/R_1 = 1.0^+$) (i.e., R_1^+ is R_1 at the membrane side as distinguished from R_1, which is R_1 at the floc side), Eq. (4) can be written using Eqs. (7) and (9) in the following form:

$$f(p_{\omega 1^+}) = C_1 - C_2/\omega_1 = f(p_{\omega 2}) + R_2 \cdot Kg_p (p_b - p_{\omega 2}) - (R_2^2/R_1) Kg_p (p_b - p_{\omega 2})$$

Thus,

$$f(p_{\omega 1^+}) = f(p_{\omega 2}) + R_2 \cdot Kg_p (1 - R_2/R_1)(p_b - p_{\omega 2}) \tag{10}$$

At the interface between the membrane and the floc (the point ω_1^-) we have:

$$Dp_f \, dp / d\omega \,|\, \omega_1^- = Dp_m \cdot dp / d\omega \, \omega_1^+ \tag{11}$$

similar to Eq. (6) we have:

$$Dp_m\, dp/d\omega \mid \omega_1^+ = C_2/\omega_1^+ = C_2 \tag{12}$$

Thus from Eqs. (11), (12), and (7) we get:

$$Dp_{f_{\omega_1}-} dp/d\omega \mid \omega_1^- = (R_2^2/R_1)\, Kg_p\, (p_b - p_{\omega 2}) \tag{13}$$

If we use the one internal collocation point approximation for the diffusion-reaction equation inside the floc as shown in the next section, then the left-hand side of Eq. (13) can be approximated using the orthogonal collocation formulae (42), and thus Eq. (13) becomes:

$$Dp_{f_{\omega_1}-} *(A_{21}\, p_c + A_{22}\, p_{\omega 1}^-) = (R_2^2/R_1)\, Kg_p\, (p_b - p_{\omega 2}) \tag{14}$$

where A_{21} and A_{22} are the collocation constants for spherical shape given by (42), $A_{21} = -3.5$ and $A_{22} = 3.5$, and c indicates the internal collocation point.

Ethanol Mass Balance Inside the Floc

Unsteady state mass balance inside the floc, considering all the reactions producing ethanol and consuming sugar is taking place inside the floc we obtain the following parabolic partial differential equations:

$$\partial p/\partial t = \bar{\mu}_m \cdot \rho_d \cdot Y_p \cdot \mu'/Y_c + (1/r^2)\,(\partial/\partial r)\,[Dp_f\, r^2\, \partial p/\partial r] \tag{15}$$

putting the equation in dimensionless coordinate, $\omega \in (0, 1)$, $\omega = r/R_1$, we obtain the equation:

$$R_1^2\, \partial p/\partial t = \bar{\mu}_m R_1^2 \cdot \rho_d \cdot Y_p \cdot \mu'/Y_c + (1/\omega^2)\,(\partial/\partial\omega)\,[Dp_f\, \omega^2\, (\partial p/\partial\omega)] \tag{16}$$

Appling the orthogonal collocation technique using one internal collocation point at ω_c we obtain at the internal collocation point the following differential equation:

$$R_1^2\,(dp_c/dt) = B_{11}\,[Dp_{fc} \cdot p_c] + B_{12}\,[Dp_{f\omega 1}^- \cdot p_{\omega 1}^-] + \Phi_s^2 \cdot Y_p \cdot \mu' \cdot [S_c, p_c] \tag{17}$$

where, B_{11}, B_{12} are given as (38): $B_{11} = -10.5$, $B_{12} = 10.5$

Equations (10), (14), and (17) are two algebraic equations and one differential equations in four variables, namely, $p_{\omega 2}^+$, $p_{\omega 1}^-$, $p_{\omega 1}$, p_c, thus one equation is missing. This extra equation can be furnished by the equilibrium relation between $p_{\omega 1}^+$, $p_{\omega 1}^-$, which has the following form (43):

$$p_{\omega 1}^- = K_1 \cdot p_{\omega 1}^+ \tag{18}$$

Equations (10), (14), (17), and (18) can be solved to obtain the four variables at every time during the fermentation process, provided at these times the concentration of the sugar at the collocation point is known (the necessary equations are derived in the next section) and the bulk (extracellular) concentrations are known (the extracellular equations are exactly the same as in ref. (1) and are given in a later section). The solution algorithm as a whole is given in Appendix (8).

Mass Balance for Sugar Inside the Membrane

We assume no reaction taking place inside the membrane and also assume pseudo-steady state for the membrane. Furthermore, we assume constant sugar diffusivity through the membrane. Based on these assumption the sugar material balance inside the membrane becomes:

$$\nabla^2 S = 0 \tag{19}$$

where,

$$\nabla^2 = d^2/d\omega^2 + (2/\omega)\,(d/d\omega),\ \omega = r/R_1$$

The boundary condition at the outer surface of the membrane ω_2 $(=R_2/R_1)$ is given by,

$$dS / d\omega \mid \omega_2 = Sh_s\,(S_b - S_{\omega 2}) \tag{20}$$

where,

$$Sh_s = Kg_s \cdot R_1/Ds_m$$

The boundary condition at the inner surface of the membrane ω_1 $(=R_1/R_1=1.0)$ is given by:

$$Ds_f\, dS / d\omega \,|_{\omega 1}{}^- = Ds_m\, dS / d\omega \,|_{\omega 1}{}^+ \tag{21}$$

The general solution of Eq. (19) is given by:

$$S = C_1' - C_2'/\omega \tag{22}$$

After some manipulation we obtain $S\omega_2$ in terms of $S\omega_1{}^+$:

$$S_{\omega 2} = [S_{\omega 1}{}^+ + Sh_{sm} \cdot S_b\,(1 - R_1/R_2)] \,/\, [1 + Sh_{sm}\,(1 - R_1/R_2)] \tag{23}$$

where,

$$Sh_{sm} = Sh_s * (R_2/R_1)^2$$

The boundary condition in Eq. (21) coupled with the floc differential equation after simplifying it using the one-internal orthogonal collocation point approximation, gives the following relation between $S_{\omega 1}{}^-$, $S_{\omega 1}{}^+$ and the concentration of sugar at the internal collocation point, Sc:

$$S_{\omega 1}{}^- = \{Sh_{sf}\,(S_b - S_{\omega 1}{}^+) \,/\, A_{22}\,[1 + Sh_{sm}\,(1 - R_1/R_2)]\} - (A_{21}/A_{22}) \cdot S_c \tag{24}$$

where,

$$Sh_{sf} = Sh_{sm} * (Ds_m/Ds_f)$$

The change of sugar concentration at the internal collocation point is given by the following nonlinear differential equation:

$$(R_1^2/Ds_f)\, dS_c / dt = B_{11}\, S_c + B_{12} \cdot S_{\omega 1}{}^- - \Phi_s^2 \cdot \mu'\,(S_c,\, p_c) \tag{25}$$

where,

$$\Phi_s^2 = \rho_d \cdot \bar{\mu}_m \cdot R_1^2 \,/\, Ds_f\, Y_c,$$

$$\mu' = [K_p \cdot S(1-X/X_m)^N / (K_p+p)(K_s+S)] + [K_p' / (K_p' + p)]$$

In addition we assume the validity of the equilibrium relation between $S_{\omega 1}^-$ and $S_{\omega 1}^+$ written as:

$$S_{\omega 1}^- = K_2 \cdot S_{\omega 1}^+ \tag{26}$$

The Extracellular Balance Equation for the Batch Fermentor Case

The extracellular mass balance equations are the same as those in ref. (*1*):

For sugar:

$$dS_b / dt = [-3X \cdot Kg_s / (\rho_d - X)R_2](S_b - S_{\omega 2}) \tag{27}$$

For ethanol:

$$dp_b / dt = [-3X \cdot Kg_p / (\rho_d - X)R_2](p_{\omega 2} - p_b) \tag{28}$$

For the microorganisms:

$$dX / dt = \bar{\mu}_m \cdot \mu' \cdot X \tag{29}$$

Equations (10), (14), (17), (18), and (23–29) are the model equations that are to be solved together with the physical properties external mass transfer and diffusivities correlations to obtain the change of sugar, ethanol, and microorganisms concentrations with time.

For the fed-batch case, the above equations are modified to account for the feeding rate period as shown in ref. (*1*).

RESULTS AND DISCUSSION

The model has been solved using the algorithm given in Appendix (8), the physical parameters estimations given in Appendices (1–7), and the kinetics and physical parameters given in ref. (*1*). The thickness of membrane is taken as 10^{-6} cm for the two cases studied (one is an experimental batch fermentation of Novak et al. (*2*) and the second is an industrial fed-batch fermentor). The results of the present model are compared with the experimental and industrial results as well as the results of the previous model in ref. (*1*), where the ethanol mass transfer coefficient has been lumped into one parameter, which was estimated by empirical fitting to one set of Novak et al.'s (*2*) experimental results as a function of sugar concentration.

Figure 2 shows the experimental intracellular ethanol concentration profile vs time together with the profile predicted by the model in ref. (*1*) (model 1) and the profile predicted by the present distributed parameter

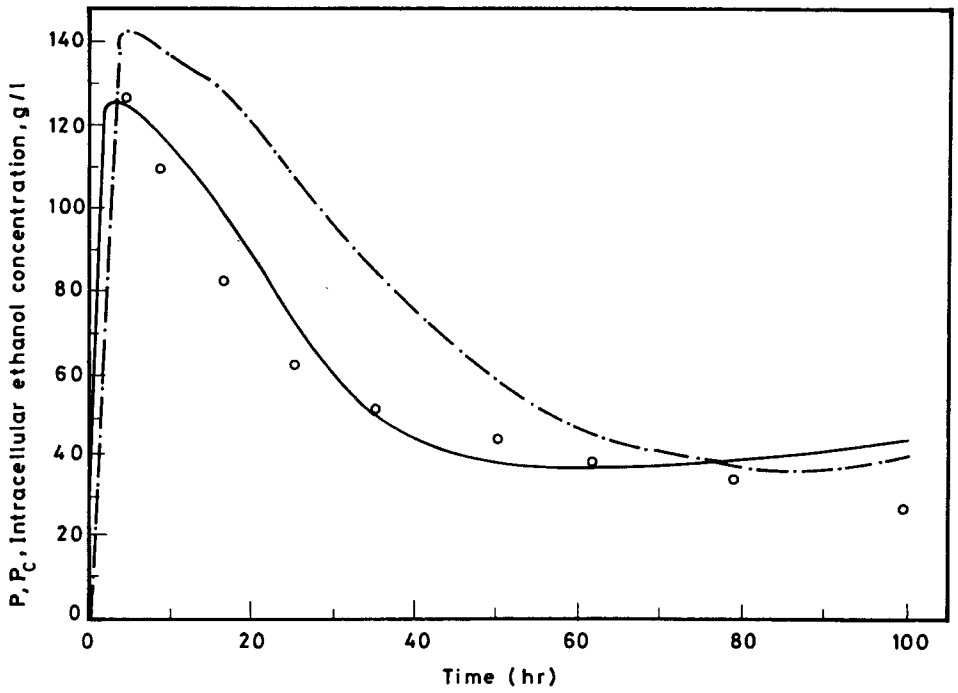

Fig. 2. Intracellular ethanol concentration vs time. ○: experimental; ——: model 1 (empirical Kg_p); —·—: distributed parameter model. Batch experimental fermentor.

model (DPM) at the internal collocation point. The results of the present (DPM) model gives the nonmonotoinic shape of the intracellular ethanol concentration and the profile as a whole follows the same experimental trend, however the deviation from the experimental results is larger than that of model (1).

Figure 3 shows the profiles of the extracellular sugar concentration. The results of the (DPM) are very close to the experimental results and the results of model (1).

Figure 4 shows the extracellular ethanol concentration profiles where the (DPM) predicts ethanol concentrations that are very close to the experimental results and the prediction of model (1).

Figure 5 shows the yeast concentration profiles, the (DPM) predict yeast concentrations that are lower than the experimental values and the predictions of model (1).

Figure 6 shows the extracellular sugar concentration profiles for the industrial fed-batch fermentor. The (DPM) gives results that are very close to the results of model (1) (model [1] was checked successfully against the industrial results as presented in ref. [*1*]). The difference between the predictions of the two models increases slightly at the last 4 h of fermentation.

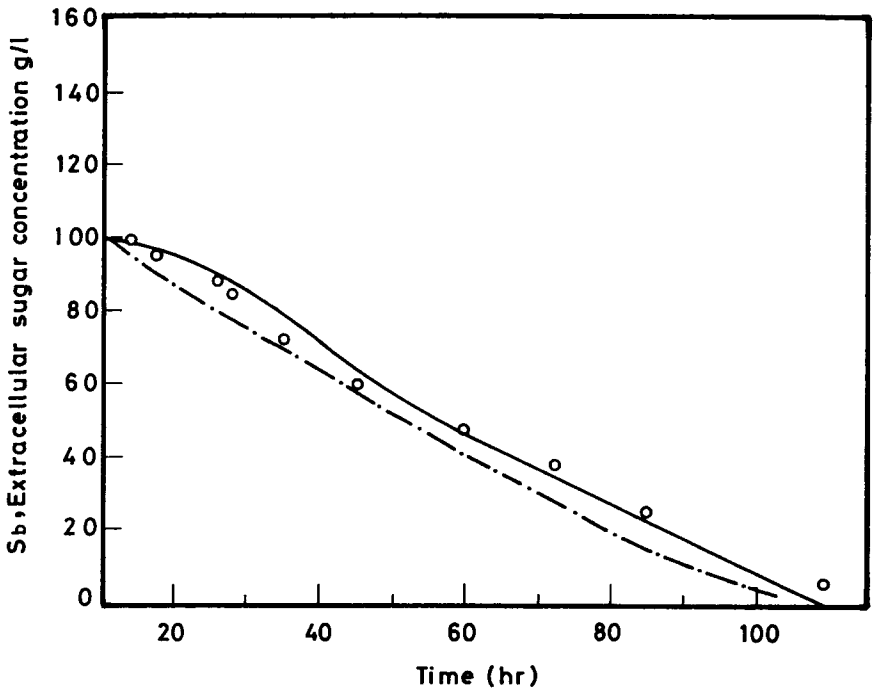

Fig. 3. Extracellular sugar concentration vs time. ○: experimental; ——: model 1; —·—: distributed parameter model. Batch experimental fermentor.

Figure 7 shows the extracellular ethanol concentration profiles. The results of the (DPM) are very close to those of model (1). The difference between the predictions of the two models increases slightly at the last 3 h of fermentation.

Figure 8 shows the yeast concentration profiles. The difference between the predictions of the two models is in the order of 20%.

CONCLUSIONS

Although in the present model the estimation of the different mass transfer resistances includes a number of gross assumptions, the results of the model as compared with the experimental and industrial results are reasonably good. The results of the model with empirical K_{gp} (*1*) gives better results, however it suffers from the limitation of having one of the parameters K_{gp} estimated empirically from a certain set of experimental results (2). The present model, although including a number of assumptions, opens the road for further improvements by developing more relia-

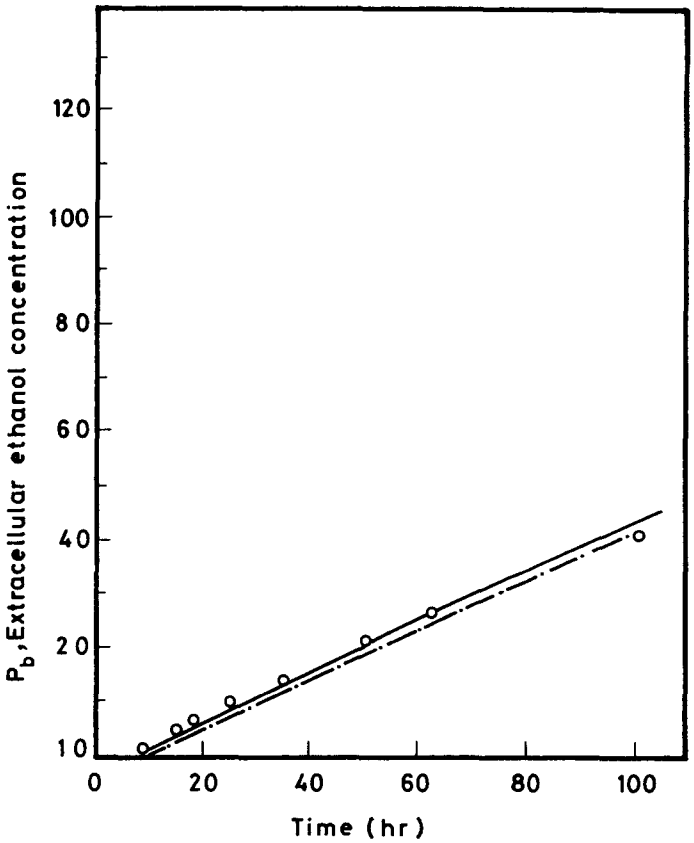

Fig. 4. Extracellular ethanol concentration vs time. ○: experimental; ——: model 1; —·—: distributed parameter model. Batch experimental fermentor.

ble correlation for the estimation of the different mass transfer resistances and relaxing some of the assumptions through a well directed coupled experimental and modeling research program. The present model offers deeper insight into the process of mass transfer and biochemical reaction during fermentation. Thus promising to be of more general value than the model with empirical K_{gp} and therefore helps in directing research efforts toward developing more rigorous models with less assumptions and uncertainties based on a deeper understanding of the complex diffusion-reaction processes taking place in the system. The main research issues for the further development of the model are: (1) gaining a better understanding of the diffusion process taking place through the membrane; and (2) using a more detailed (structured) model for modeling the internal of the floc.

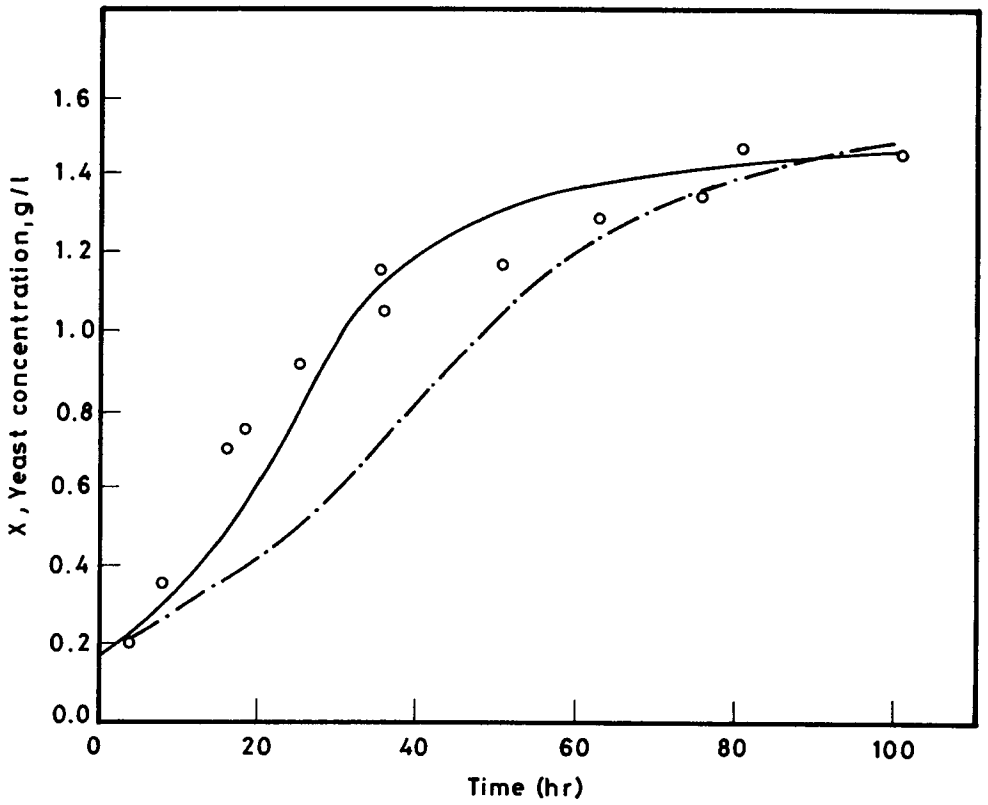

Fig. 5. Yeast concentration vs time. ○: experimental; ——: model 1; —·—: distributed parameter model. Batch experimental fermentor.

APPENDIX 1

The External Mass Transfer Resistances (Resistances Between the Extracellular Fluid (S_p, P_b) and the External Surface of the Membrane ω_2)

The external mass transfer coefficients Kg_p and Kg_s are estimated from Calderbank's correlation (*44*):

$$Kg \cdot dp / D = 2 + 0.3\, Sc^{1/3} [(\rho_\omega - \rho)\, dp^3 \cdot \rho \cdot g/\mu^2] \qquad \text{(A-1)}$$

where, $Sc = \mu/\rho \cdot D$, dp = floc diameter, D = diffusivity, Kg = mass transfer coefficient, ρ_ω = floc density, ρ = fluid density, g = acceleration of gravity, and μ = liquid viscosity.

The mass transfer coefficients Kg_p and Kg_s are computed from relation (A-1) and these values changes with the time of fermentation due to the change in physical parameters (particularly μ, ρ), with the change of the medium properties due to the transformation of substrate to ethanol. The dependencies of these parameters on the change of composition during fermentation are given in Appendices (2–4).

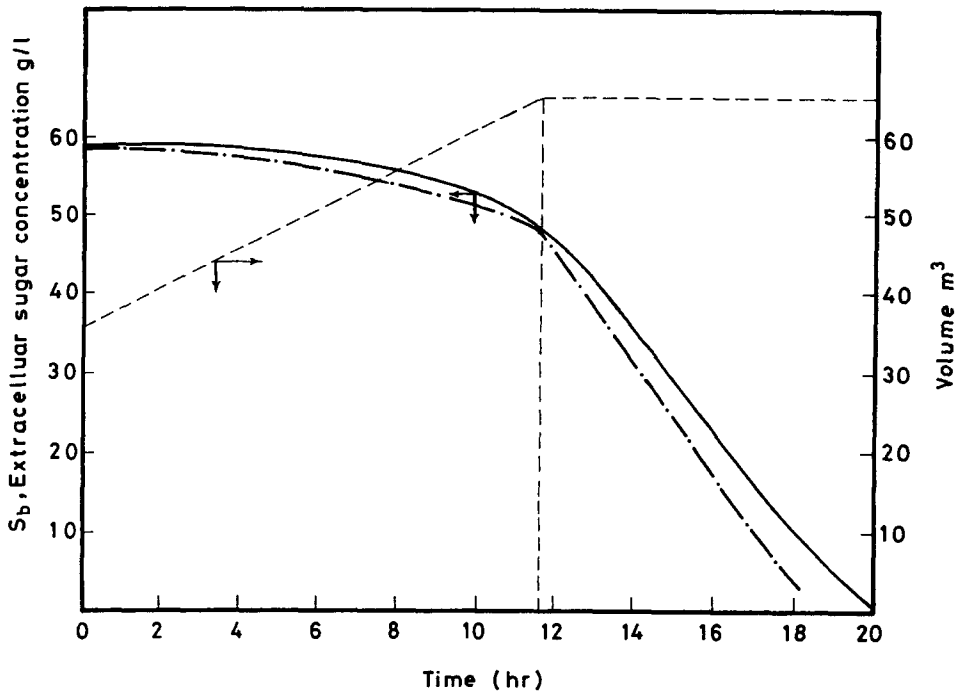

Fig. 6. Extracellular sugar concentration vs time. ——: model 1; —·—: distributed parameter model. Fed-batch industrial fermentor.

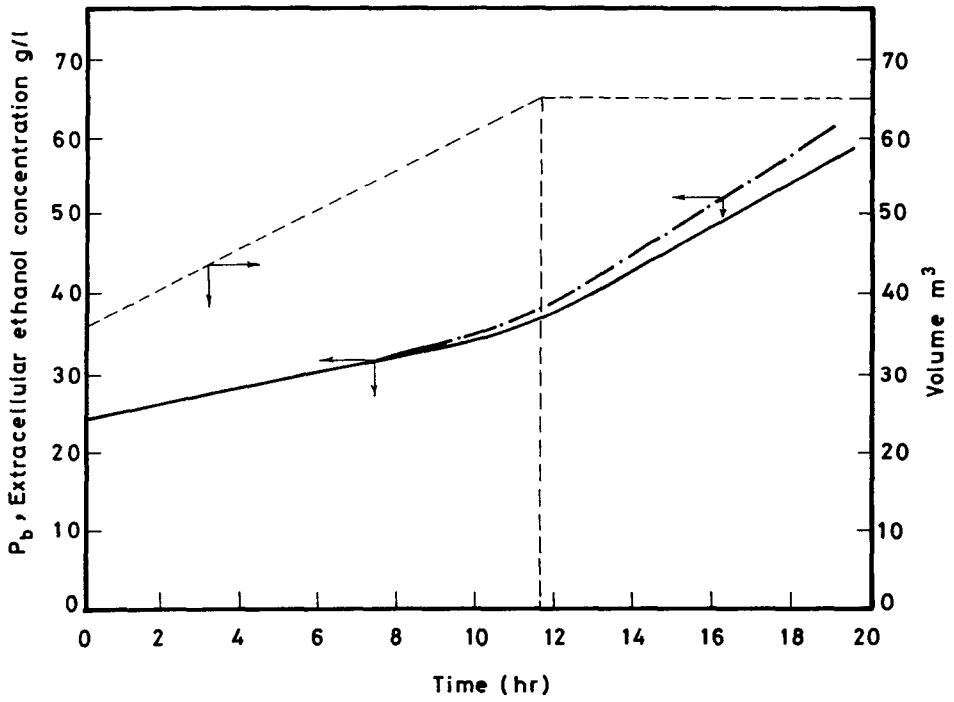

Fig. 7. Extracellular ethanol concentration vs time. ——: model 1; —·—: distributed parameter model. Fed-batch industrial fermentor.

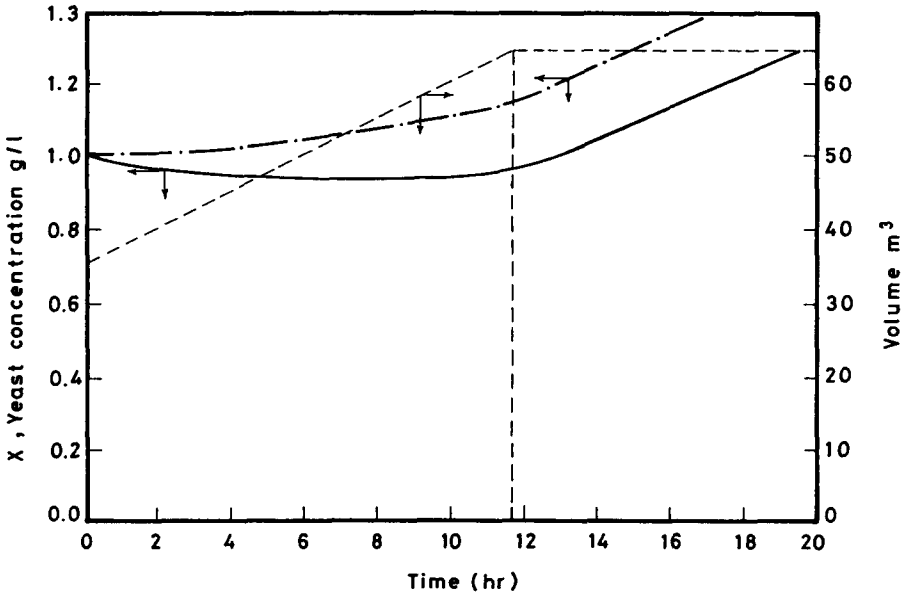

Fig. 8. Yeast concentration vs time. ——: model 1; —·—: distributed parameter model. Fed-batch industrial fermentor.

APPENDIX 2

The Change in Solution Density with the Progress of Fermentation

Kollerup and Daugulls (*45*) correlated the solution density variation with the sugar and ethanol concentrations by the equation:

$$\rho = 1000 + \alpha_1 \cdot S_b + \alpha_2 \cdot p_b \qquad \text{(A-2)}$$

where,

$$\alpha_1 = 0.411,\ \alpha_2 = -0.163 \text{ and } S_b,\ p_b \text{ are in g/L}$$

APPENDIX 3

The Change in Solution Viscosity with the Progress of Fermentation

The viscosity of sugar solution varies with the concentration of sugar in the solution according to the relation (*45,46*):

$$\mu_s = 0.8007 + 0.003047\, S_b \tag{A-3}$$

whereas the viscosity of an ethanol solution change with ethanol concentration according to the relation (*45*):

$$\mu_p = 1.801126 - 7.503742 * 10^{-4}\, p_b \tag{A-4}$$

S_b and p_b are bulk concentration of sugar and ethanol respectively in g/L.

The viscosity of the mixture of ethanol and sugar can be computed from the Kendall-Monroe relation (*47,48*):

$$(\mu_m) = (x_1\, \mu_1^{1/3} + x_2\, \mu_2^{1/3})^3 \tag{A-5}$$

where x_1 and x_2 are the mol fractions of components 1, 2 respectively.

For our system the viscosity of the mixture is thus given by:

$$\mu_m = (x_1 \{\mu_{\text{ethanol}}\}^{1/3} + (1-x_1) \{\mu_{\text{glucose}}\}^{1/3})^3 \tag{A-6}$$

where x_1, which is the mol fraction of ethanol in the extracellular solution, is given by:

$$x_1 = (p_b/M_E) / [(S_b/M_s) + (p_b/M_E)] \tag{A-7}$$

where M_E = mol wt of ethanol and M_s = mol wt of glucose.

APPENDIX 4

Diffusion Coefficients of Sugar and Ethanol in Solution

The diffusion coefficients of glucose in water and ethanol in water are given by (*49*):

$$Dp_{at}\ 25\ C = 1.28 * 10^{-5}\ \text{cm}^2/S \tag{A-8}$$

and,

$$Ds_{at}\ 25\ C = 6.9\ * 10^{-6}\ \text{cm}^2/S \tag{A-9}$$

Correction for temperature and the different viscosity of the solution can be carried out using the relation (*49*):

$$D_2 = (\mu_1/\mu_2)\cdot(T_2/T_1)\cdot D_1 \tag{A-10}$$

where state 1 is 25C for water solution, whereas state 2 is 30C for the solution of glucose and ethanol with composition that is varying with conversion. This completes the necessary information for the computation of Kg_s, Kg_p variation with conversion.

APPENDIX 5

Estimation of Ethanol Diffusivity Through Membrane

The diffusivity of either ethanol or sugar through the membrane were computed using the following procedure. The diffusivities in solution can be estimated from the Wilke-Change relation (*50*):

$$D = 7.4 * 10^{-8} \cdot [(\Phi M)^{1/2}/V_o^{0.6}\mu] \cdot T = \alpha_{22}/\mu \qquad \text{(A-11)}$$

where, D is the mutual diffusion coefficient of solute A at very low concentrations in solvent B, cm^2/S; V_o is the molar volume of solute at normal bp cm^3/g mol; M is mol wt of solvent at normal bp; Φ is association parameter; μ is viscosity of solvent C.P.; T is absolute temperature K.

The membrane of the yeast cell consists of lipids, proteins, and polysaccharides containing mannose (*49*).

Fatty acids are relatively simple lipids, however lipids also constitute proteins of more complex molecules such as lipoproteins and liposaccharides, which also appear in cell membrane.

Typical percentage in baker's yeast (*saccharomyces cerevisiae)* are 6–8% protein including several enzymes and about 30% of glucan and about 30% D-glucose.

Avakyants (*51*) found that for wine yeast, the percentage of fatty acids (C_{12}–C_{24}) in lipids is from 30–60%. James et al. (*52*) found that glucose polymer β-glucan is the most abundant polysaccharide occurring in the cell wall of yeast comprising about 12–14% of the dry wt of whole cell (according to their chemical analysis glucan represents 36–39% of the cell wall). Obviously the diffusion process through this membrane is a very complex process.

In the present model we assume that the diffusion of ethanol through the membrane occurs only in the saturated and unsaturated C_{16} fatty acyl acid that varies during the course of fermentation as shown by Sullivan et al. (*53*) and shown in Table A-1.

We also consider that the fatty acid (C_{16}) and ethanol occupies completely the pores of the membrane.

For the parameters of equation (A-11) we take $\Phi = 1$, $V_o = 58$, M (mol wt of C_{16} fatty acyl) $= 244$, $T = 303K$. The viscosity will depend on the concentration of ethanol in the pores according to the relation:

$$\mu_m = (x_1 \{\mu_{\text{ethanol}}\}^{1/3} + x_2 \{\mu_{\text{fatty acid}}\}^{1/3})^3 \qquad \text{(A-12)}$$

where,

$$x_1 = (p/M_E) / \{[(\rho_\omega - p)/M_{\text{fatty acid}}] + p/M_E\},\ x_2 = 1 - x_1 \qquad \text{(A-13)}$$

Table A-1
Percentage of Saturated and Unsaturated Fatty Acyl C_{16} in the Lipid of *S. Cerevisiae* Membrane, During the Course of Batch Fermentation

Fermentation time (h)	Unsaturated fatty acyl C_{16}:% of total lipid	Saturated fatty acyl C_{16}:% of total lipid	Total fatty acyl C_{16} % of total lipid
8	43.6	24.1	67.7
16	32.8	39.0	71.8
32	25.8	46.1	71.9
64	26.9	41.7	68.6

In the above formulation for the mol fraction we considered the cell membrane to be formed of fatty acid plus ethanol with ρ_ω being considered the density of the cell wall (wet wt/wet vol).

APPENDIX 6

Estimation of Glucose Diffusivity Through the Membrane

There are strong evidences that glucose uptake limits the rate of fermentation in *S. Cerevisiae* (*54,55*), however the mechanism of glucose uptake in yeast is not completely understood (*56*). Some investigators explain the increased glucose uptake in immobilized yeast cells by the proposition that immobilization damages the cell wall and thus destroys its resistance to diffusion (*57,58*). In the present paper we model the glucose uptake as a diffusion process although there are some evidences that it may be an active transport process (see the discussion of this point in ref. [*1*]).

Onuma et al. (*59*) correlated the diffusion coefficient of glucose in biomass as a function of the carbon to nitrogen ratio $R = C/N$ and temperature as follows:

$$D_{st}/D_{s20} = (1.008)^{(t-20)} * \exp[-6.79*10^{-5}\cdot\rho_d\cdot R] \quad \text{(A14)}$$

where,

$$D_{s20} = 6.47 * 10^{-6}\ \text{cm}^2/\text{Sec} \quad \text{(A-15)}$$

at $t = 30C$, the relation becomes:

$$D_{s30} = (1.008)^{10}(D_{20})\cdot\exp[-6.79 * 10^{-5} * \rho_d\cdot R] \quad \text{(A-16)}$$

The carbon to nitrogen ratio R for *S. Cerevisiae* is equal to 6 (*60*).

APPENDIX 7

Estimation of the Diffusion Coefficient of Glucose and Ethanol Inside the Floc

For glucose we assume that the diffusion coefficient inside the floc is the same as in the membrane. For ethanol we use the same relations (A-11), (A-12) after replacing the fatty acid with the cytoplasm thus using the mol wt and viscosity of cytoplasm instead of that of the fatty acid.

For the viscosity of the cytoplasm we take it as a function of sugar concentration inside the floc:

$$\mu_{cyt} = 1 + 85.0 * S \qquad \text{(A-17)}$$

whereas the mol wt of cytoplasm is taken as: $M_{cyt} = 300$.

APPENDIX 8

The Solution Algorithm

For the given initial bulk conditions at $t = 0$, p_{bo}, S_{bo} and the initial conditions for p_c, S_c the solution algorithm is as follows:

1. Equations (10), (14), and (18) together with the relations for physical properties and diffusivities given in the Appendices are solved to obtain $p_{\omega 2}$, $p_{\omega 1}{}^{+}$, $p_{\omega 1}{}^{-}$.
2. Equations (23), (24), and (26) together with the relations for physical properties and diffusivities given in the Appendices are solved to obtain $S\omega_2$, $S\omega_1{}^{+}$, $S\omega_1{}^{-}$.
3. The differential Eqs. (17), and (25) for the concentration at the internal collocation point are integrated using a 4th order Runge-Kutta routine with automatic step size (to ensure accuracy) to obtain p_c, S_c for the next time step.
4. The differential Eqs. (27), (28), and (29) are integrated using the same routine to obtain p_b, S_b, X for the next time step.
5. Repeat 1–4 till you reach the final time of fermentation.

APPENDIX 9

The Constants of Eq. (4)

$$A = b^3/d^3,\ B = (3b^2/d^3)\,(a\cdot d - c\cdot b),\ A_1 = (3b/d^3)\,(a\cdot d - c\cdot b)^2,$$
$$A_2 = (1/d^3)\,(a\cdot d - c\cdot d)^3$$

where,

$$a = R_M\cdot\rho_\omega,\ b = 1 - R_M,\ c = (\mu_{\text{lipid}})^{1/3}\cdot R_M\cdot\rho_\omega,$$
$$d = (\mu_{\text{ethanol}})^{1/3} - R_M\cdot(\mu_{\text{lipid}})^{1/3}$$

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