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DIFFUSIONAL PROCESSES IN THE BIOMASS CONVERSION

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Abstract

Diffusional process with biochemical reaction was investigated. Fermentation for ethanol production with *Saccharomces Cerevisia* was carried out with Ca-alginate gel in the form of layer spherical beads in the anaerobic conditions. The kinetic parameter determination was achieved by fitting reaction progress curves to the experimental data. The diffusion coefficients determination was performed for experimental conditions. The obtained results shows effect the biochemical reaction rate and diffusion.

Keywords: Ethanol production; diffusion rate; transfer coefficient; immobilized cell; spherical bed.

1. Introduction

Diffusional substance transfer rate has been investigated by many authors [1-3]. Substance transfer phenomena of the bioactive substance between the solid and liquid phases was studied in previous papers [1,4-12]. Transfer phenomena in penicillin fermentation were investigated by Savkovic-Stevanovic et.al., and Vico-Stevanovic et.al.[6-7].

In this paper the biomass conversion in ethanol was investigated taking into account bioreaction rate and diffusion rate in anaerobic conditions. Substrate and product mass and heat transfer rate were examined.

2. Material and heat transfer rates

Taking into account diffusion only in the *x* direction, microscopic description phase transfer phenomena in the fluid flow can be described (Fig.1).

Material balance for substrate,

$$\frac{\partial c_s}{\partial t} + v_z \frac{\partial c_s}{\partial z} + (-r_s)(\frac{1}{\varepsilon_b}) = D_{efS}(\frac{\partial^2 c_S}{\partial x^2}) + k_L a_S (c_S - c_S^*) \quad (1)$$

Material balance for product,

$$\frac{\partial c_P}{\partial t} + v_z \frac{\partial c_P}{\partial z} + (r_P)(\frac{1}{\varepsilon_b}) = D_{efP}(\frac{\partial^2 c_P}{\partial x^2}) + k_L a_P (c_P - c_P^*) \quad (2)$$

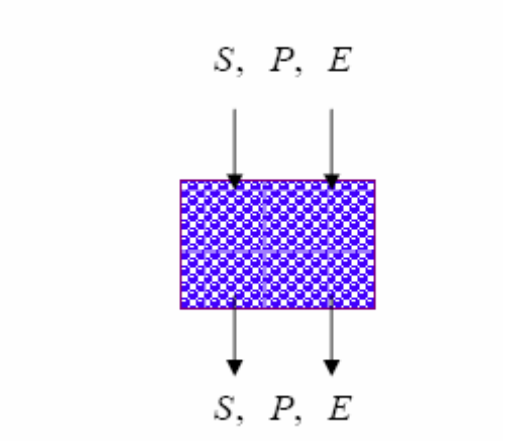

Fig.1 A packed bed

For the spherical particle:
Material balance for substrate,

$$\frac{\partial c_s}{\partial t}+v_z\frac{\partial c_s}{\partial z}+(-r_s)(\frac{1}{\varepsilon^p})=D_{efS}(\frac{\partial^2 c_S^p}{\partial r^2}+\frac{2}{r}\frac{\partial^2 c_S^p}{\partial r}) \quad (3)$$

and product:

$$\frac{\partial c_P}{\partial t}+v_z\frac{\partial c_P}{\partial z}+(r_P)(\frac{1}{\varepsilon^p})=D_{efP}(\frac{\partial^2 c_P^p}{\partial r^2}+\frac{2}{r}\frac{\partial^2 c_P^p}{\partial r}) \quad (4)$$

Heat balance:

$$\rho C_p(\frac{\partial T}{\partial t}+v_z\frac{\partial T}{\partial z}+(-r)\Delta H)=\lambda(\frac{\partial^2 T}{\partial x^2}) \quad (5)$$

The derived mathematical models can be used for microscale simulation of the ethanol production.

3. The biochemical reaction rate

General kinetic model have involved Monod's model and Michaelis-Menten's with taking into account inhibition [6-9].

$$E+S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} P+E \quad (6)$$

$$\frac{dc_{ES}}{dt}=k_1\cdot c_E\cdot c_S-k_{-1}\cdot c_{ES}-k_2\cdot c_{ES} \quad (7)$$

and product rate

$$r_p=\frac{dc_P}{dt}=k_2\cdot c_{ES} \quad (8)$$

where E is enzyme, S is substrate, ES-enzyme complex, P is product, c is concentration and k is specific biochemical rate constant $k=A_0e^{-E_a/R_gT}$.

4. Substrate and product diffusion

The effective diffusivities of the glucose and the ethanol were determined. The equations (9) and (10) were used for the diffusion coefficients calculation. Their solution has given concentration's profile inside particle bead are:

$$c_S(t)=c_{L0}\frac{a}{a+1}\{1+\sum_{n=1}^{\infty}\frac{6(1+a)\exp[-D_{efS}q_n^2(t/R^2)])}{9+9a+q_n^2a^2}\frac{a}{r}\frac{\sin[q_n(r/R)]}{\sin q_n}\} \quad (9)$$

where c_S substrate concentration inside particle, c_{L0} initial concentration solution, a= liquid volume/ particle volume, R is particle diameter, r is distance from particle center, t is time and q positive root of the equation $\tan q_n = 3q_n/(3+aq^2{}_n)$.

The effective diffusion coefficient was calculated using the closed pore model for a spherical bead [3-5].

$$c_L(t)=c_{L0}\frac{a}{1+a}\{1+\sum_{n=1}^{\infty}\frac{6(1+a)\exp(-D_{efS}q_n^2(t/R^2)}{9+9a+q_n^2a^2}\} \quad (10)$$

where c_L substrate concentration which can be measured. Diffusion coefficient was determined by fitting curve with experimental data by the Levenberg-Marquardt method.

5. Experimental data

Fermentation for ethanol production with *Saccharomces cerevisia* was carried in the anaerobic conditions. The biocatalyst by cell immobilization in Ca-alginate gel in the form of two layer spherical beads were used. The chosen two layer bead decreases cell wash from the gel preserving uniform cell distribution in the gel. A two layer bead was designed in order to increase the biocatalyst operational stability. The packed bed column provides high cell concentration in the bioreactor and decreases the rate of cell wash by increased mixing efficiency. Experimental set up shows in Fig. 2.

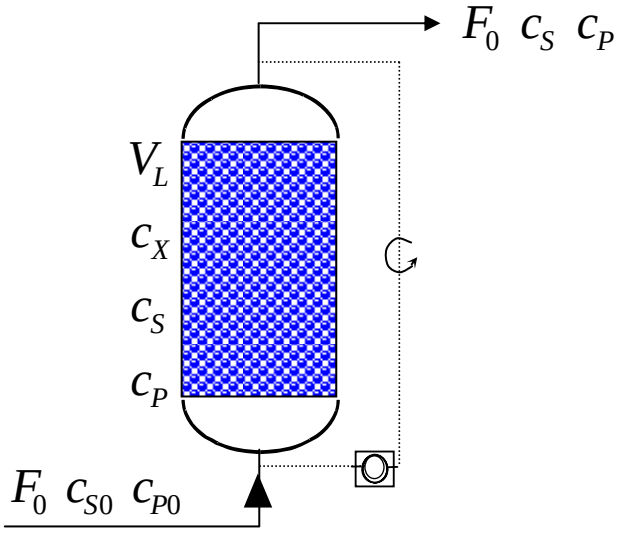

Fig.2 A bioreactor scheme

The packed bed column provides high cell concentration in a bioreactor and decreases the rate of cell wash by increased mixing efficiency. The immobilization efficiency and biocatalyst activity were investigated by diffusion and reaction rate. Inhibition of the fermentation caused by high substrate and product concentrations was examined.

6. Substance transfer coefficients

The overall component transfer coefficients were defined involving outside limited diffusion, inter phase transfer and inner diffusion.
For substrate,

$$\frac{1}{k_{LS}a_S} = \frac{1}{D_{OS}} + \frac{1}{(\delta / D_{lS})} + \frac{1}{D_{inS}} \tag{11}$$

and product

$$\frac{1}{k_{LP}a_P} = \frac{1}{D_{OP}} + \frac{1}{(\delta / D_{lP})} + \frac{1}{D_{inP}} \tag{12}$$

The component transfer coefficients are defined. The effective substrate and product transfer coefficients which taking into account specific reaction rate constants and effective component diffusion coefficient can be coupled. According to eqs. (6) - (8) biochemical parameter can be defined as:

$$k_M = (k_{-1} + k_2) / k_1 \tag{13}$$

Then, effective glucose and ethanol transfer coefficients can be defined:
For substrate glucose,

$$\frac{1}{k_{LS}a_S} = \frac{1}{(\frac{1}{D_{efS}})(k_1 - k_{-1})} \tag{14}$$

and product ethanol,

$$\frac{1}{k_{LP}a_P} = \frac{1}{(\frac{1}{D_{efP}})k_2} \tag{15}$$

7. Parameters optimization

When a process is defined, the result is the solution of the governing equations at particular values of the input variables and parameters. Often, the values of these parameters are known imprecisely. The effect of these errors or uncertainties is generally of concern to the design engineer, and if may be desirable to quantify these errors. One method of quantification involves calculation of the sensitivity coefficients of the process, which are matrices of partial derivatives of the form:

$$\partial(solution)/\partial(parameter) \quad (16)$$

The magnitude of the dimensionless log-normalized sensitivity,

$$\frac{\partial \ln x_i}{\partial \ln \alpha_j} = \frac{\alpha_j}{x_i}\frac{\partial x_i}{\partial \alpha_j} \quad (17)$$

is indicative of the relative importance of the parameter α_j to the solution x_i. Quantitatively, the log-normalized sensitivities give a percentage change in the parameter values.

In the frame of this investigation parameter determination was achieved by fitting process progress curves to the experimental data. It is the goal of regression analysis or curve fitting to obtain values of unknown parameters in an equation utilizing experimental data [13]. Regardless of wether the equation is linear or nonlinear in the parameters, a criterion for determining the best model parameters is required. This requirement is commonly satisfied by the least squares objective function.

$$s = \sum_{i=1}^{N}(y_i - y_i')^2 \quad (18)$$

where y_i' is predicted or calculated value of the dependent variable, by regression equation, for the *i*-th observation, y_i is experimental value of the dependent variable for the *i*-th observation and *N* is number of experimental points.

The best values of the model parameters are obtained when the objective function is minimized. Methods for estimating the constants may be divided in two categories linear methods and nonlinear methods. The nonlinera methods are iterative in nature, i.e. starting values are picked and upgraded by the algorithm until a convergence criterion is satisfied. For linear models, the calculational procedure is direct and thus an iterative procedure is not required.

Each of these methods can also be used for root location problems, system of algebraic equations by setting $y_{i=}0$ in the objective function and $y_i'=f_i(x)$ where $f_i(x)$ is the algebraic function of the unknown independent variables *x*. Also, some of the general optimization methods described elsewere in this text may be effective for least squares objective functions [14].

Integral minimization criteria based of the least squares was implemented. Further, kinetic parameters data base was used for bioprocess optimization.

The biochemical specific constants were determined and parameters adequateness and significance were tested by Fisher's and student's t-test. Sensitivity analysis of the parameters was performed. The Levenberg-Marquardt optimization method was used.

8. Results and discussion

Rate of the glucose consumption for various initial substrate concentration has shown in Fig.3. Fig. 4 shows product formation rate for various substrate initial concentration and initial biomass concentration c_{X0} =3.5 g/dm^3.

The obtained results for effective diffusion coefficients are shown in Table 1 - 5. and Figs.5-7. The effective substrate and product transfer coefficients were determined. The obtained values for glucose and ethanol are shown in Figs. 7 and 8.

The determination specific reaction rate constants were achieved by fitting bioreaction progress curves to the experimental data. The obtained values were tested by Fisher's statistical test. Specific rate constants have show in Table 6.

Table 1. Ethanol diffusion coefficients for various cells concentration in the gel particle (a= 4.2 m^2/m^3, d_p=3.0mm, alginate gel (DM/V)=2%, temperature=28°C)

%(SM/V)	D_e m^2/s	Linearity, r
1	10.700 10^{-10}	1
2	9.897 10^{-10}	1
3	9.506 10^{-10}	1
4	9.107 10^{-10}	1

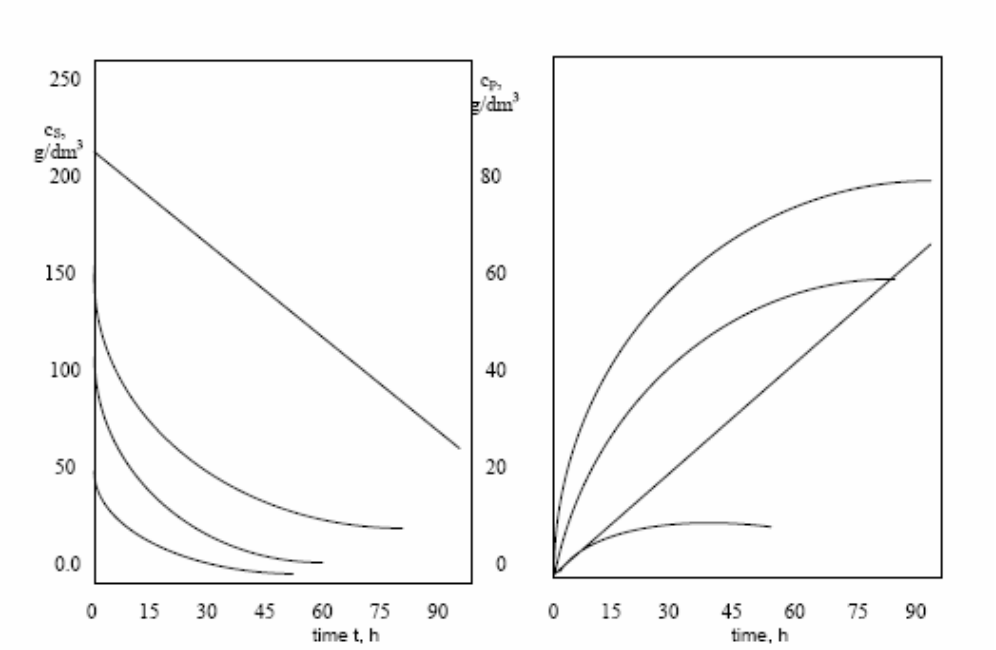

Fig.3 Glucose consumption for initial substrate concentration (50-250) and c_{X0}=3.5 g/dm^3

Fig.4 Ethanol formation rate for initial substrate concentration (50-250) and c_{X0}=3.5 g/dm^3

Table 2. Glucose diffusion coefficients for various initial substrate concentration c_{So} (a= 4.2 m^2/m^3, d_p=3.0mm, alginate gel (DM/V)=2%, temperature=28°C and c_{X0} =3.5 g/dm^3)

c_S g/dm^3	D_e m^2/s	Linear correlation, r
50	6.202 10^{-10}	0.992
100	6.202 10^{-10}	0.992
150	6.191 10^{-10}	0.992
200	6.202 10^{-10}	0.992

Table 3. Glucose diffusion coefficients for various Ca-alginate gel fraction (a= 4.2 m^2/m^3, d_p=3.0mm, temperature=28°C)

%(DM/V)	D_e m^2/s	Linearity, r
1	6.402 10^{-10}	1
2	6.200 10^{-10}	1
3	9.000 10^{-10}	1
4	5.800 10^{-10}	1

Table 4. Glucose diffusion coefficients for various cell concentration in the gel particles (a= 4.2 m^2/m^3, d_p=3.0mm, temperature=28°C)

%(VM/V)	D_e, m^2/s	Linearity, r
10	5.420 10^{-10}	1
20	5.010 10^{-10}	1
30	4.201 10^{-10}	1
40	3.689 10^{-10}	1
50	2.750 10^{-10}	1

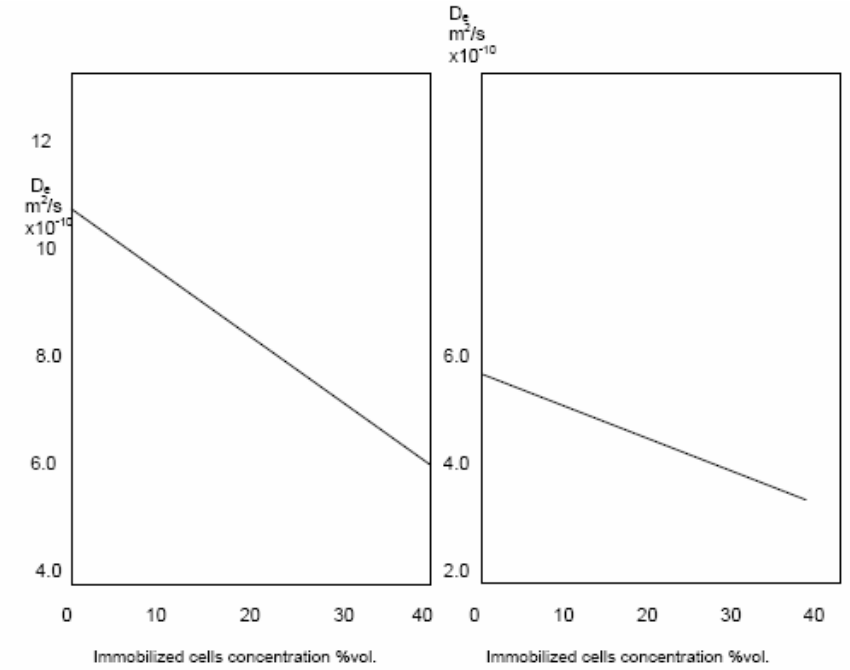

Fig.5 Effective ethanol diffusion coefficients vs. immobilized cell concentration

Fig.6 Effective glucose diffusion coefficients vs. immobilized cells concentration

Table 5. Glucose diffusion coefficients for various particles diameters (a= 4.2 m^2/m^3, d_p=3.0mm, temperature=28°C)

d_p, mm	D_e, m^2/s	Linearity, r
1	6.209 10^{-10}	1
2	6.202 10^{-10}	1
3	6.200 10^{-10}	1
4	6.201 10^{-10}	1

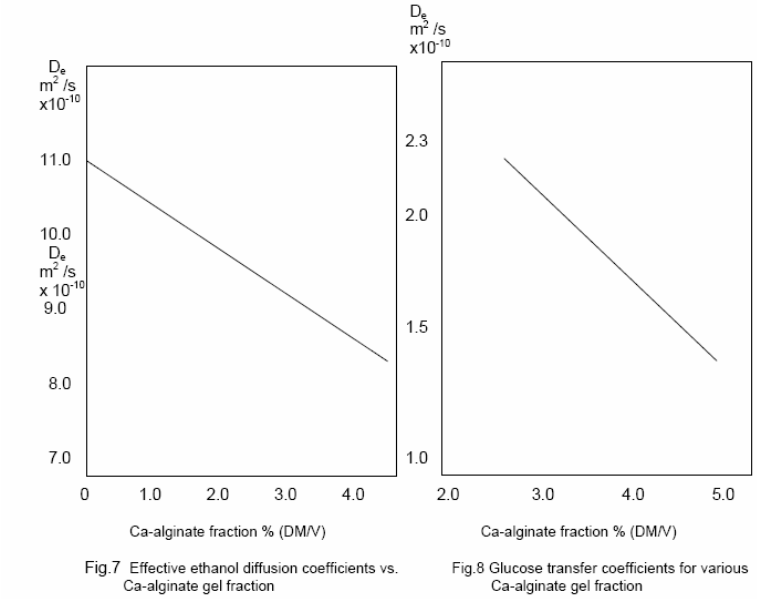

Fig.7 Effective ethanol diffusion coefficients vs. Ca-alginate gel fraction

Fig.8 Glucose transfer coefficients for various Ca-alginate gel fraction

Table 6. Specific rate reaction constants with immobilized cells for various substrate concentration (c_{Xo} =3.5 g/dm^3)

c_S g/dm^3	k_1	k_{-1}	k_2
50	0.923	0.282	0.902
100	1.093	0.516	0.945
150	1.275	0.596	0.806
200	0.864	0.377	0.680

9. Conclusion

In this paper diffusional processes with biochemical reaction were studied. The packed bed column provides high cell concentration in a bioreactor and decreases the rate of cell wash by increased mixing efficiency. The immobilization efficiency and biocatalyst activity were investigated by diffusion and reaction rate. The component diffusion coefficients and specific rate constants were determined. Parameters optimization was achieved by fitting process progress curves to the experimental data.

The glucose and ethanol transfer coefficients were determined, using effective diffusion coefficients and specific rate constants for immobilized biocatalyst.

The obtained results in this paper can be applied in the others biosystems domain.

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Notation

A_0-acceleration factor
c- concentration, g/dm^3
c_{L0} -initial concentration solution, g/dm^3
D-diffusion coefficient, m^2 /s
DM/V-dry mass per volume fraction
E_a-energy activation, J/mol
E-enzyme
F-Fisher's statistical test parameter
k-specific reaction rate constant
k_La– volumetric mass transfer coefficient, m^2 /s
q- positive root of the equation $tan\ q_n = 3q_n/(3+aq^2_n)$
R_g-gas constant, J/mol degree
r_{cor} -correlation coefficient
r_S- substrate reaction rate
r_P- product reaction rate
R is particle diameter, mm

r is distance from particle center, mm
t-time, h
V_L - void volume of the fermentation medium in the bioreactor,dm^3
WM/V-wet mass per volume fraction

Greek symbols

a- liquid volume/ particle volume
δ **-** layer thickness, mm
η -effectiveness factor
ε **-** porosity $\varepsilon = \varepsilon^p\ (1-\varepsilon_b) + \varepsilon_b$

ε^p - particle porosity

ε_b – biocatalyst layer porosity

λ **-**thermal diffusivity

Superscript

p-particle

Subscription

L-liquid
l –bounded layer
P- product
S- substrate
X-biomass

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