INTERACTION OF HYDRODYNAMIC ENVIRONMENT ON PERFORMANCE OF HOMOGENEOUS BIOREACTORS WITH ENZYME KINETIC MODELS

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Abstract

Interaction of hydrodynamic effects on enzyme reactions in homogeneous non-ideal industrial bioreactors is in the work from theoretical and computational point of view analyzed. The results are extended to the case of unstructured model of a chemostat with an extracellular product synthesis. Considered are the cases of two limiting hydrodynamic states: 1) total segregation or macromixing; 2) complete or micro mixing. The effects of hydrodynamic conditions are represented by assumed experimentally determined residence time distribution curve. For the case of complete molecular mixing Zwietering's life expectancy function is applied. For macromixing state conversions are determined by convolution of residence time distribution function with conversion functions from batch kinetics. Studied are effects of degree of mixing on efficiency of Michaelis-Menten kinetics without inhibition and with substrate inhibition (negative order kinetics). Chemostat is modeled with unstructured Monod based kinetics for biomass growth and substrate consumption. Theoretical evolution of the residence time distributions is derived from various combinations of perfectly stirred vessels and ideal plug flow in tubes. The aim of the work is to establish practical guidance for estimation of lower and upper bounds for bioconversions in nonideal industrial reactors.

Key words: micromixing, residence time distribution, enzyme kinetics

Introduction

Modelling complex hydrodynamic behaviour is one of the most difficult numerical problems, but at the same time it is of fundamental importance to many aspects of biochemical engineering. It requires solution of Navier-Stokes equation with supplied rheological and/or turbulence models coupled with heat and mass balances. A numerical solution is attainable by CFD - computational fluid dynamic methods that are based on extensive space and time meshing and use of finite element approximation of solution of Navier-Stokes equation. From reactor engineering aspect, hydrodynamic effects in a reactor are due to flow, pumping and impeller rotation in vessels, and for

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continuous bioreactors are usually described on macroscopic scale by probability residence time density function RTD, (1,2,3). However, hydrodynamic effects on chemical and biochemical reaction on microscopic scale and knowledge of RTD function does not suffice for evaluation of a bioreactor performance (4). In this work a numerical method based on mass balances is applied for the boundary cases of the earliest and the latest micromixing. The boundary cases of micromixing correspond to maximum mixidness and segregated flow respectively. On macroscopic scale, the model of "tanks in series" is applied for numerical evaluation of RTD function.

Mathematical model

Modelling of hydrodynamics effects based on RTD experiments can be simplified in view of assumed flow patterns in a nonideal (industrial) bioreactor. Number of model parameters varies from zero to a case with several adjustable model parameters subject to statistical fitting procedure with data from a tracer impulse response measurement and theoretical model. The zero parameter models are the limiting cases corresponding to segregated and maximum mixidness flow. Models with one adjustable parameter are derived form visualisation of a flow pattern like in a tank in series or as a dispersion flow in a tubular reactor. Models with more adjustable parameters are constructed by assuming a complex flow as in system of interconnected vessels. Most commonly is applied RTD function *E(θ)* of the tank in series of equal volume perfectly mixed tanks is obtained by the inverse of Laplace transform of the product of *N* individual transfer functions:

$$E(\theta) = \mathrm{L}^{-1}\left[\prod_{i=1}^{i=N}\left(\frac{1}{\frac{1}{N}\cdot s+1}\right)\right] \tag{1}$$

In Eq. (1). RTD is expressed as function of dimensionless time θ defined as the ratio of real time and the average residence time, which equals to the ratio of a vessel volume and volumetric flow rate through the vessel. The function is model for a very broad spectrum of RTD functions ranging from ideally continuous stirred tank reactors (CSTR), various packed bed reactors, and in the limiting case for $N \to \infty$ it corresponds to a response of a plug flow in a tubular reactor. In Fig. 1. are depicted typical RTD profiles obtained by Eq. (1).

For given RTD function uniquely is defined the life expectancy function LEF as function of the expectancy variable (also named "intensity function"), Λ (λ), given by:

$$\Lambda(\lambda) = \frac{E(\lambda)}{1 - \int_0^{\lambda} E(\theta)\cdot d\theta} \tag{2}$$

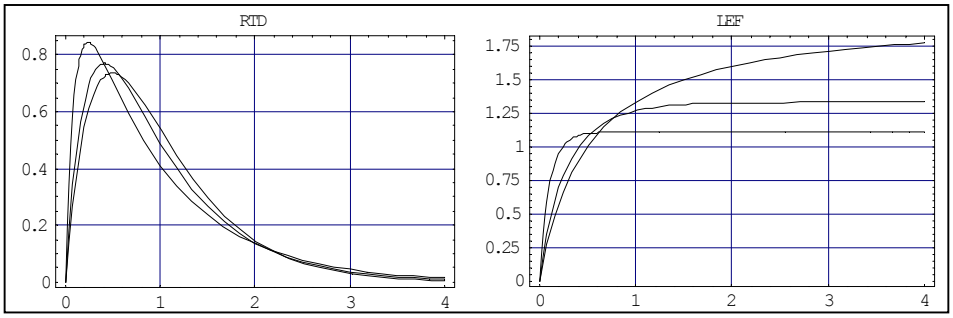

Figure 1. Residence time distributions RTD and Life expectancy function LEF for tank in series model for the following variance of residence times: 0.1; 0.2; and 0.5.

Mass balances for reacting species in a bioreactor under batch operating conditions are expressed in the matrix form:

$$\frac{d}{d\theta}\vec{c} = Da \cdot \boldsymbol{\alpha}^T \cdot \vec{r}(\vec{c}) \qquad (3)$$

Stoichiometric matrix for a set of reactions is given by α, and volumetric reactions are given as components of the vector $\vec{r}$ given by generalised Michaels-Menten kinetic expressions. Applied is the relative time scale leading to introduction of Damköhler dimensionless number defined as the ratio of maximum rate of reaction and maximum rate of convection through reactor. The balances (3) apply for intracellular and extracellular species derived from homogeneous and/or structured models of microorganism metabolism. However, it is more meaningful when volumetric rates *r* are replaced by specific rates *v* expressed per unit dry mass of cells:

$$\vec{r} = \vec{v}(\vec{c}) \cdot c_X \qquad (4)$$

The balances (3) are integrated for given initial conditions $c(0) = c_0$ and a specified *Da*, yielding the solutions $c(\theta, Da)$. The initial concentrations for batch balances (3) are identical to concentrations in a feeding stream in case of continuous flow through reactor. Effects of micromixing on bioreactor performance are related to degree of reaction of enzyme rates.

When enzyme reaction rate is inhibited by substrate, order of reaction rate changes from 1-st order (n=1) in the concentration range bellow saturation constant $c_s << K_s$ to negative 1-st order (n = -1) for high concentrations $c_s >> K_s$ (Fig. 2). Degree of mixidness effects the reaction rate only in the concentration region *B* where the order of reaction is $-1 < n < 1$. In the region *A* degree of mixing has no effect on reaction rate, while in region *B* increase in degree of mixidness results in increase of reaction rate. For given experimentally determined RTD function effect of degree of mixidness on enzymatic rates can be bracketed into the region defined by the bounds evaluated for zero mixidness (segregated flow) and maximum mixidness. Due to the range of order of reaction, the upper bound on conversion is evaluated by the maximum mixidness case, and the lower bound of conversion corresponds to segregated flow. The bounds are calculated based on solution of balances for reacting species under batch conditions. The balances (3) are integrated for given initial conditions $c(0) = c_0$ and a specified *Da*, yielding the solutions $c(\theta, Da)$. The initial con-

centrations for batch balances (3) are identical to concentrations in a feeding stream in case of continuous flow through reactor. For the limiting case of completely segregated flow, which for given RTD distribution can be interpreted as the case of the latest mixing, mass balance in steady state is given by the convolution of RTD and concentration profile:

$$\vec{c} = \int_0^\infty E(\theta) \cdot \vec{c}(\theta, Da) \cdot d\theta \tag{5}$$

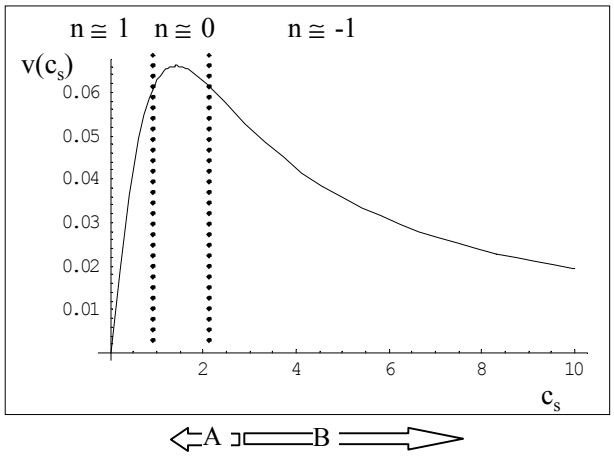

Figure 2. Change of order n of enzyme reaction rate with substrate inhibition corresponding to substrate concentration regions

The opposite boundary case is defined by assumption of the earliest mixing, when the maximum mixidness is achieved. In this case the mass balances involve the life expectancy function and are evaluated by a set of nonlinear differential equations:

$$\frac{d}{d\lambda}\vec{c}(\lambda) = -Da \cdot \boldsymbol{\alpha}^T \cdot \vec{r}(\vec{c}) + \Lambda(\lambda) \cdot \left(\vec{c}(\lambda) - \vec{c}_0\right) \tag{6}$$

The asymptotic right hand boundary condition is applied:

$$\frac{d}{d\lambda}\vec{c}\ (\lambda)\Big|_{\lambda=\infty} = 0 \tag{7}$$

The solution of the steady state problem is given by the initial condition:

$$\vec{c}_S = \vec{c}(\lambda = 0, Da) \tag{8}$$

Integration of (6) has to be evaluated in the reverse direction (from right to left) with the initial condition determined from the nonlinear algebraic equation:

$$0 = -Da \cdot \boldsymbol{\alpha}^T \cdot \vec{r}\left(\vec{c}, \lambda = \infty\right) + \Lambda(\infty) \cdot \left(\vec{c}\left(\lambda = \infty\right) - \vec{c}_0\right) \qquad (9)$$

In view of the relative time scale, when mean residence time is equal to 1, the limiting value for $\lambda \to \infty$ is replaced by a number for which RTD is practically negligible, for example for $\lambda = 4\,(or\,8)$. The nonlinear problem (9) requires an iterative procedure such as Newton algorithm, and since system of differential equation is "stiff" and requires backward integration, it has to be executed with a robust ODE integrator, such as LSODE.

Results

The model for effects of degree of mixing on performance of continuously operated bioreactors is analysed. The problem is motivated by the importance of evaluation of boundaries of conversion (or productivity) based only on RTD information without prior knowledge of actual degree of mixidness, or the need for solution of Navier-Stokes equation by CFD software. The marginal error is determined for a range of Damköhler numbers in a practical region, from the washout state when conversion is approaching zero, to the upper values of Damköhler number for which conversion is approaching 100 %. In view of applicability of simulation results it is essential to have the evaluation in the full space of parameters, which is accomplished by rending the problem in dimensionless form. The dimensionless form of the model equations also improves numerical stability of integration algorithm.

Investigation of RTD on performance of bioreactors has been of a continuous interest to biochemical engineers working with non-Newtonian liquids in reactors of large volumes (5,6)**,** in cases of complicated flow patterns occurring in open containers for biodegradation of polluted waters (7), or in multiphase aerated bioreactors (8,9,10,11). In this work performance of chemostat, i.e. continuously and isothermally operated liquid phase bioreactor with suspended microorganisms, is investigated. The balances are accounted by the rate limiting substrate (y) and biomass (z). Dimensionless balances in the batch mode of operation, corresponding to Eq. (3), are:

$$\frac{d}{d\theta} y = -\beta \cdot Da \cdot \mu(y) \cdot z \qquad (10)$$

$$\frac{d}{d\theta} z = Da \cdot \mu(y) \cdot z \qquad (11)$$

$$\mu(y) = \frac{y}{\gamma_S + y + \frac{y^2}{\gamma_I}} \qquad (12)$$

The initial conditions are provided by the dimensionless values of the feeding concentrations $y(0)=1$ $z(0)=1$. The process kinetics is given by the dimensionless growth function (13) for which the kinetics order changes in the range from + 1 to -1 with increase of substrate concentration *y*. This information is sufficient for proving that maximum mixidness will provide the upper bound for substrate conversion, while the segregated flow correspond to the lower bound. In Fig. 3 are presented results obtained for the following set of parameters: γ_S =1, γ_I =10, β = 0,8, and variance of RTD $\sigma^2(\theta)$ = 0,5. The software *Mathematica* v. 4.0.(12) has been used for numerical evaluation of the substrate and biomass balances (5-6).

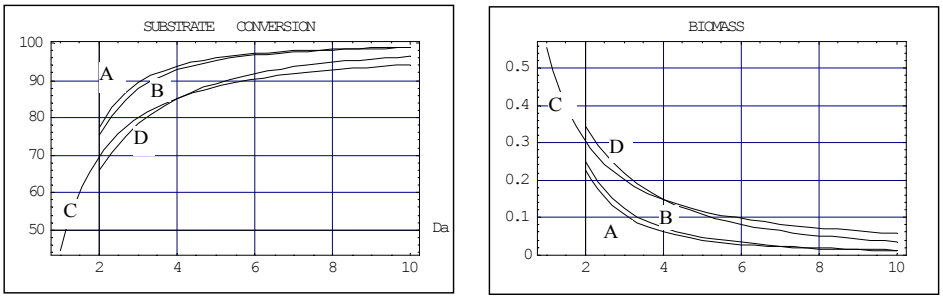

Figure 3. Substrate and biomass concentrations as function of Damköhler number at following mixing conditions: A) maximum mixidness; B) true (test case). C) ideally mixed; D) segregated flow.

The batch mode balance (3) is evaluated numerically by use of *NDSolve* with *Da* as a variable parameter. For the segregated balance (5) numerical integration by adaptive Gauss quadrature is applied as provided by the *NIntegrate* program. The nonlinear equation for determination of the missing right hand boundary value is calculated by Newton algorithm as given in *NSolve*. The critical step is the backward integration of the nonlinear set of stiff equations (6). Applied is the powerful adaptive integrator *NDSolve* which encompasses LSODE algorithm. In order to provide analysis of degree of mixidness at a range of *Da* number, the integrals are defined as a function *Da*. On Fig. 3- are also shown results obtained from the test problem (exact solution), and the case for assumed ideally mixing (ideal chemostat). The errors for the upper and lower bound with respect to the exact solution are also given in Table 1.

Table 1. Comparison of errors in substrate conversion for the upper and lower bound of mixidness

	Percentage error in substrate conversion	
Da	upper bound error maximum mixidness	lower bound error segregated flow
2	2,077	-9,477
4	1,049	-7,701
6	0,444	-4,926
8	0,223	-3,303
10	0,128	-2.345

The results prove that for mixing in bioreactors defined by the test model of "tanks in series" the maximum mixidness model will provide very accurate upper bound on substrate conversion for the whole practical range of Damköhler number. Maximum error of the upper bound is +2 %

at the point close to "washout", and with further increase of *Da* the error decreases to 0,1 % when conversion is practically 100%. Errors of the lower bound are for a degree off order larger.

Conclusions

Evaluation of conversions in a nonideal biochemical reactor requires solution of the numerically intensive problems of mixing in bioreactors based on Navier-Stokes equation and application of the computational fluid dynamics (CFD). The problem can be effectively approximated by evaluation of upper and lower bounds of conversions based on the assumptions of maximum mixidness and segregation. The maximum mixidness provides the upper bound while the segregated yields the lower bound for substrate conversions. However, for a chemostat without inflow of biomass the lower bound calculated by segregated flow is zero (plug flow chemostat does not sustain biomass).

For tested models of RTD derived from tanks in series model the upper bound gives small error $\Delta < +2$ %, and is decreasing with increase of *Da* value. Maximum error for the lower bound is $\Delta < -10$ %, and is also decreasing with increase of *Da* value.

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