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Stability and response analyses of phenol degrading biochemical systems

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Chemical systems having non-linear dynamic state equation exhibit unusual behaviour like multiplicity, sustained oscillations etc., when perturbation in any forcing function is somehow introduced to an existing steady state. Under this situation a reacting system may exhibit one or more ambiguous steady states. Biochemical systems using microorganisms following either substrate- or product-inhibited growth kinetics are usually characterized with this type of behaviour. In the present investigation biodegradation of phenol using *Pseudomonas putida* following substrate inhibited growth kinetics has been taken as model system. The system has been studied in a 2 dm^3 B. Braun fermenter in batch and continuous modes of operation separately. Using the experimental data obtained in batch mode of operation the kinetic parameters of the systems have been determined considering substrate inhibited Haldane type kinetics for biodegradation of phenol. Two separate techniques, *viz*., linear stability analysis and phase plane analysis have been performed to study the characteristics of steady states from local and global points of view respectively. Response analysis of the system has been performed using step-type perturbations in inlet substrate concentrations of different amplitudes. The experimental findings show good agreement with the simulated results.

Keywords: Phenol biodegradation, Bioreactors, Stability analysis, Dynamic simulation, Mathematical modeling

Stability and consistency are the key characteristics concerned with the most efficient operation of bioreactors. Control of bioprocesses is challenging due to the high degree of nonlinearity involved in the dynamics of biochemical systems. As evident from the reported works[1-14], biochemical systems often exhibit unusual and uncontrollable dynamic behaviour like oscillatory transition from one steady state to another and sustained oscillation, very long time period to reach a steady state and multiplicity, *i.e.,* the occurrence of multiple steady states corresponding to a single set of values of process parameters, similar to auto thermal processes. Therefore, an a-priori knowledge of stability behaviour of bioreactors is necessary to avoid such undesired situations. Although several investigations have been done on the control characteristics of biochemical systems, more intense and systematic works substantiated by experimental findings in the specific fields of application of bioprocesses are still in need in this area. As the abatement of pollution caused by organic substances is one of the burning problems for which bioprocesses may provide green routes, it is intended under the present study to analyze an effluent treatment bio-process, characterized with peculiar instability behaviour, as a model bio-system from the perspectives of stability and response analyses. It is already established that microbial route is one of the potential ones for the removal of phenol occurring in the liquid effluent stream of many industries based on organic chemicals[15-17]. The phenol degrading bio-systems are usually characterized by substrate inhibition. Moreover, the presence of parametric sensitivity with respect to system *p*H is another peculiarity of this system, which has thoroughly been dealt with by Lallai & Mura[17] and Dutta *et al*[18]. Thus, it is apprehended that phenol degrading bio-systems may be susceptible to instability causing difficult control situation. No work has, so far, been reported on the process control criteria of this peculiar bio-system characterized with parametric sensitivity. Therefore, in the present investigation a comprehensive and qualitative analysis of the stability criteria of biodegradation of phenol using *Pseudomonas putida* culture following *p*H sensitive substrate inhibited Haldane kinetics have been carried out. While local stability analysis has been performed by linear analysis, general stability of the bio-system has been studied by phase plane analysis using the concept of separatrices introduced by Blanch and Clark[8]. These analyses are expected to help in identifying different zones according to their stability characteristics in a vector/ parameter space with the dilution rate and the inlet substrate concentration as

the main input variables. Keeping in mind that general biodegradation kinetics of the present system is a well studied area and also keeping in mind the seminal contribution of Kumar *et al*.[15], it is felt that it is still necessary to investigate the basic biodegradation kinetics of phenol since the intrinsic kinetic parameters are of utmost important for the later stage of analysis *viz*., stability and response.

It is well understood that even if the appropriate values of process parameters with respect to stability criteria are set in a bioreactor, because of unpredictable upsets due to fluctuations in pumping rate, flow patterns, inlet substrate concentration, control action is needed to maintain specified conditions. Therefore, in the present work, real time response analysis has been done by studying system dynamics in the time domain in response to step perturbations of different amplitudes. Dilution rate (D) and the inlet substrate concentration ($C_{A,in}$) have been used as the forcing functions. The experimental observations are in good agreement with the simulated time domain responses.

Moreover, non-ideality of the bioreactor has been assessed using two-parameter model, i.e., ideal reactor with bypassing and dead volume[11,19]. Tracer technique is used to evaluate the parameters in the model.

Experimental Procedure

Microorganism

Microorganism used in the study of biodegradation of phenol was *Pseudomonas putida* obtained from National Collection of Industrial Microorganism (NCIM), Pune, India.

Reactor

Fermentation using phenol as substrate was carried out in a 2 dm^3 B.Braun fermenter (Biostat ®B2) using *Pseudomonas putida.* The fermenter was an autoclavable glass vessel (W×H×D mm: 305×580×270) with double jacket, stainless steel lid and baffle cage. The reactor was equipped with open temperature control system for the maintenance of constant operating temperature throughout the process. A 180 watt electronic motor was provided with the reactor for stirring the culture medium in the range of 50-1200 rpm.

Medium

Stock cultures of *Pseudomonas putida* were maintained in nutrient agar medium consisting of beef extract (10 g/dm^3), NaCl (5 g/dm^3), peptone (10 g/dm^3) and agar (20 g/dm^3) as prescribed by NCIM. Stocks were stored at 4°C. All chemicals, unless otherwise stated, were of A. R. grade.

Acclimatization procedure

Working cultures of *Pseudomonas putida* were developed as follows. The culture was grown aseptically in a phenol limiting nutrient medium (Phenol: variable, $(NH_4)_2SO_4$: 0.5 g/dm^3, $Mg(SO_4)_2.7H_2O$: 0.1 g/dm^3, $FeCl_3.6H_2O$: 0.0005g/dm^3, $CaSO_4.2H_2O$: 0.03g/dm^3, NaCl: 0.05 g/dm^3, KH_2PO_4: 0.527 g/dm^3, Na_2HPO_4: 0.873 g/dm^3, tap water 0.1 dm^3, distilled water to volume) in a 2 dm^3 B. Braun fermenter at a temperature of 30°C[16]. The *p*H of the medium has been fixed depending on the purpose of the study. The acclimatization procedure was performed in three subsequent stages. In the three stages the initial concentration of phenol was varied from 0.1 to 0.15 g/dm^3. The duration of each stage was 86.4 ks.

Batch studies for determination of kinetic parameters for phenol degradation

To study the kinetics of the biodegradation of phenol by *Pseudomonas putida* in batch mode, several experimental runs were carried out in the 0.1 dm^3 Erlenmeyer flask varying the concentration of the limiting substrate, *i.e.,* phenol, in the range of 0.06-0.280 g/dm^3. The flasks were placed in incubator cum shaker where the temperature of the culture broth was maintained at 30±1°C. Programmed experiments were carried at three pre-set and controlled values of system *p*H (5, 6 and 7) for each initial concentration of phenol to study the effect of *p*H on the reaction rate. Samples were drawn periodically from the fermented broth and were analyzed for both phenol and cell mass concentrations.

Flow system

Experiments in continuous mode were also conducted in 2 dm^3 B. Braun fermenter under aerobic condition. The bioreactor was fed with sterile medium using a peristaltic pump. The liquid product was also taken out of the fermenter at the same rate using another peristaltic pump. The reactor was initially filled with 1 dm^3 medium and was inoculated with the acclimatized culture. Dilution rate was varied in the range of 0.0278 to 0.0833 ks^{-1} and the inlet concentration of limiting substrate *i.e.,* phenol, was varied in the range of 0.06-0.280 g/dm^3. The *p*H of the culture broth was maintained at 7.0. The stirrer speed and the temperature of the culture broth were the same as those maintained during batch studies.

Tracer experiment

Potassium permanganate, the tracer, was injected in the system through step input. The tracer concentration in the effluent stream has been measured as a function of time using spectrophotometric method. Thus, residence time distribution (RTD) of the non-reacting substance i.e., tracer was determined experimentally.

Analytical method

Biomass concentrations were measured by dry weight method. The culture broth was centrifuged at 81.667 rps for 0.6 ks. The cells were washed twice with distilled water. Aluminium cups containing the cell mass were left for 86.4 ks at 80°C for drying. Finally the weight of cell mass was taken. Phenol concentrations were determined by the 4-amino-antipyridine method[20].

Theoretical analysis

Model equations for flow reactor

Under real situation, the chemostat may not behave ideally due to the generation of dead space, bypassing of a fraction of reacting stream without conversion etc. To account for the probable non-ideality in the chemostat, analysis of reactor behaviour has been done both from ideal and non-ideal perspectives.

Ideal behaviour study

The biochemical system chosen for the present investigation is biodegradation of phenol using *Pseudomonas putida*. Simulation of continuous type fermenter has been carried out using the following data and assumptions:

1 As the biochemical process takes place in an ideal chemostat, no spatial variation of parameters exists.
2 Physicochemical properties, *viz.*, density, viscosity etc., of the reaction broth do not change with the propagation of reaction.

The material balance equations for substrate and biomass under steady state chemostat are as follows:

$$D\left(C_{\mathrm{A,in}} - C_{\mathrm{AS}}\right) - \frac{\mu C_{\mathrm{BS}}}{Y_{\mathrm{X/S}}} = 0 \qquad \ldots(1)$$

$$D\left(C_{\mathrm{B,in}} - C_{\mathrm{BS}}\right) + \mu C_{\mathrm{BS}} = 0 \qquad \ldots(2)$$

Degradation of phenol using biochemical route follows Haldane type substrate inhibited kinetics[17]. For such system, coupling the polynomial functionality of system *p*H with Haldane type kinetics[16,18], μ can be represented as follows:

$$\mu = \left(\frac{\mu_{\mathrm{m}} C_A}{K_{\mathrm{S}} + C_{\mathrm{A}} + \frac{C_{\mathrm{A}}^2}{K_1}} \right) \left(c_1 p\mathrm{H}^2 + c_2 p\mathrm{H} + c_3 \right) \qquad \ldots(3)$$

The values of μ_m, K_S and inhibition constant (K_1) for phenol degrading biochemical system have been evaluated by the nonlinear regression analysis using experimental data obtained in batch mode of operation[18]. These are shown in Table 1. The expressions of coefficients c_1, c_2 and c_3 are given below,

$$c_1 = -\alpha_1 C_{\mathrm{A}} + \beta_1 \qquad \ldots(4)$$

$$c_2 = \alpha_2 C_{\mathrm{A}} - \beta_2 \qquad \ldots(5)$$

$$c_3 = -\alpha_3 C_{\mathrm{A}} + \beta_3 \qquad \ldots(6)$$

Substituting Eq. (4) - (6) in Eq. (3), one gets,

$$\mu = \left(\frac{\mu_{\mathrm{m}} \left(\delta C_{\mathrm{A}}^2 + \gamma C_{\mathrm{A}} \right)}{K_{\mathrm{S}} + C_{\mathrm{A}} + \frac{C_{\mathrm{A}}^2}{K_1}} \right) \qquad \ldots(7)$$

where,

$$\delta = -\alpha_1 p\mathrm{H}^2 + \alpha_2 p\mathrm{H} - \alpha_3 \qquad \ldots(8)$$

$$\gamma = \beta_1 p\mathrm{H}^2 - \beta_2 p\mathrm{H} + \beta_3 \qquad \ldots(9)$$

Table 1 — Values of different parameters used for biodegradation of phenol using *Pseudomonas putida*

Parameters	Values
$C_{\mathbf{A0,max}}$	0.280 g/dm^3
$C_{\mathrm{B0,min}}$	0.0534 g/dm^3
K_{I}	1.503 g/dm^3
K_{S}	0.0574 g/dm^3
α	0.95
α_1	44.0 dm^3/g
α_2	570.0 dm^3/g
α_3	1800.0 dm^3/g
β	0.05
β_1	4.2
β_2	51.5
β_3	159.0
η	0.233
μ_m	0.0889 ks^{-1}
ρ	0.36 g biomass/ g substrate

The values of α_1, α_2, α_3, β_1, β_2 and β_3 are given in Table 1.

From experimental data it has been observed that the yield coefficient $Y_{X/S}$ is dependent upon the ratio of initial biomass and substrate concentrations. The functional relationship between them may be written as follows:

$$Y_{X/S} = -\eta\left(\frac{C_{B0}}{C_{A0}}\right) + \rho \qquad \ldots(10)$$

The values of η and ρ have been determined using nonlinear regression analysis with the experimental data obtained in batch mode of operation and are given in Table 1.

On substitution of the expression for μ from Eq. (7) in Eqs (1) and (2) and noting that $C_{B,in}$=0, one gets Eqs (11) and (12) represented by

$$D\left(C_{A,in} - C_{AS}\right) - \frac{\mu_m\left(\delta C_{AS}^2 + \gamma C_{AS}\right)C_{BS}}{Y_{X/S}\left(K_S + C_{AS} + \frac{C_{AS}^2}{K_I}\right)} = 0 \qquad \ldots(11)$$

$$-DC_{BS} + \frac{\mu_m\left(\delta C_{AS}^2 + \gamma C_{AS}\right)C_{BS}}{\left(K_S + C_{AS} + \frac{C_{AS}^2}{K_I}\right)} = 0 \qquad \ldots(12)$$

Equations (11) and (12) are non-dimensionalized by introducing dimensionless parameters C^*_{AS}, C^*_{BS}, $C^*_{A,in}$, D^*.

$$D^*\left(C^*_{A,in} - C^*_{AS}\right) - \frac{\left(\delta^* C^{*2}_{AS} + \gamma C^*_{AS}\right)C^*_{Bs}}{Y^*_{X/S}\left(K^*_S + C^*_{AS} + \frac{C^{*2}_{AS}}{K^*_I}\right)} = 0 \qquad \ldots(13)$$

$$-D^* C^*_{BS} + \frac{\left(\delta^* C^{*2}_{AS} + \gamma C^*_{AS}\right)C^*_{BS}}{\left(K^*_S + C^*_{AS} + \frac{C^{*2}_{AS}}{K^*_I}\right)} = 0 \qquad \ldots(14)$$

Non-ideal behaviour study

Although a laboratory scale mixed flow reactor usually but not always behaves as an ideal reactor, in the present investigation an attempt has been made to ascertain the actual behaviour of the reactor under investigation. A detailed RTD study has been carried out using a colour tracer. In the present work the non-ideality of the reactor has been assessed using two parameter model considering the hydrodynamics of the chemostat as a combination of perfectly stirred reactor, dead space and bypass as shown in Fig. 1. Tracer experiment has been conducted under non-reacting condition for determination of the model parameters namely, α and β[19].

To determine the reactor hydrodynamics, the bypass stream and effluent stream from the reaction volume are mixed at point 2. From a balance on substrate and biomass around this point and on simplification one should get

$$C_A = \beta C_{A0} + C_{A1}(1-\beta) \qquad \ldots(15)$$

$$C_B = (1-\beta)C_{B1} \qquad \ldots(16)$$

where,

$$\beta = \frac{v_b}{v_0} = \text{Fraction of unconverted inlet feed stream.} \qquad \ldots(17)$$

For the biodegradation of phenol, mass balance of phenol and biomass on V_s, the completely stirred volume of the reactor, under steady state condition gives:

$$\frac{(1-\beta)DC_{A0}}{\alpha} - \frac{(1-\beta)DC_{A1}}{\alpha} - \frac{\mu_m C_{B1}\left(\delta C_{A1}^2 + \gamma C_{A1}\right)}{Y_{X/S}\left(K_S + C_{A1} + \frac{C_{A1}^2}{K_I}\right)} = 0 \qquad \ldots(18)$$

$$-\frac{(1-\beta)DC_{B1}}{\alpha} + \frac{\mu_m\left(\delta C_{A1}^2 + \gamma C_{A1}\right)C_{B1}}{\left(K_S + C_{A1} + \frac{C_{A1}^2}{K_I}\right)} = 0 \qquad \ldots(19)$$

where,

$$\alpha = \frac{V_S}{V} = \text{Fraction of useful volume.}$$

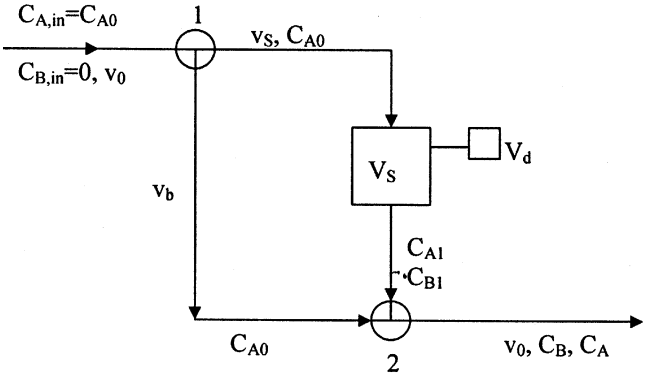

Fig. 1 — Schematic diagram of real chemostat modeled with dead space and bypass[19]

Determination of model parameters α and β

A non-reacting tracer has been injected as a positive-step input. The tracer concentration in the effluent stream has been measured at a particular time interval. The governing equation for determining the values of α and β is as follows:

$$\ln\frac{C_{T0}}{C_{T0}-C_T}=\ln\frac{1}{1-\beta}+\left(\frac{1-\beta}{\alpha}\right)\frac{t}{\tau} \qquad \ldots(20)$$

Thus, from the values of slope and intercept of the straight line obtained by plotting $\ln\frac{C_{T0}}{C_{T0}-C_T}$ against time t (figure not shown), the values of two parameters have been determined. Values of α and β have been given in Table 1.

Stability analysis

It is evident from the values of model parameters of non-ideality (α and β) that the present reactor behaves ideally. Thus, the system equations valid for ideal situation have been used for the subsequent analysis of the reactor.

The non-dimensionalized unsteady state equations for ideal chemostat are as follows,

$$\frac{dC_A^*}{d\theta}=D^*\left(C_{A,in}^*-C_A^*\right)-\frac{\left(\delta^* C_A^{*2}+\gamma C_A^*\right)C_B^*}{Y_{X/S}^*\left(K_S^*+C_A^*+\frac{C_A^{*2}}{K_I^*}\right)} \qquad \ldots(21)$$

$$\frac{dC_B^*}{d\theta}=-D^* C_B^*+\frac{\left(\delta^* C_A^{*2}+\gamma C_A^*\right)C_B^*}{\left(K_S^*+C_A^*+\frac{C_A^{*2}}{K_I^*}\right)} \qquad \ldots(22)$$

It is true that majority of biological systems follow Monod equation. However, in presence of substrate or product inhibition, the systems follow separate kinetics. The present system as presented is a clear case of substrate inhibition where Haldane equation has been proved to be the befitting kinetic model equation. Such kinetic equation which is inherently a non-monotonic equation is an interesting case for studying stability and response analysis. It is needless to mention that the system under investigation can not be explained by classical Monod equation.

It is evident that the nondimensional system Eqs (21) and (22) for phenol biodegradation representing the dynamics of the biosystem under study are highly nonlinear and therefore, are prone to show unusual behaviour, *viz.*, multiplicity, sustained oscillation etc. Thus, the stability analysis is necessary for the system from both local and global perspectives. To analyse the local stability behaviour of the system, steady state values for each operating condition have to be determined, followed by characterization of their nature by linear stability analysis. According to the concept of linear stability analysis, a steady state is stable if all the transient responses converge to it when initial conditions are situated in the vicinity of the steady state[10,11,14]. On the other hand for global/ general analysis, phase plane technique should be used to depict the overall behaviour of the systems in approaching a steady state value for preset values of system parameters, *viz.*, dilution rate and inlet substrate concentration[10,11].

For a particular feed condition the steady state values of C^*_{AS} and C^*_{BS} have been found by solving Eqs (13) and (14) using nonlinear root finding technique.

Local stability analysis

Local stability characteristics of a steady state can be performed by the method of linear analysis[10,11,14]. According to this technique, the nonlinear model equations have to be linearized first. In the present investigation, the biosystem is multivariable, nonlinear and autonomous in nature. Therefore, linearization of the model Eqs (21) and (22) have been done by considering only the first order partial derivative in the Taylor series expansion. The higher order terms in the Taylor series have been neglected with the assumption that the analysis is localized in the vicinity of the steady state under consideration. Under this condition the Jacobian matrix will be as follows:

$$\tilde{J}=\left|\begin{matrix}\frac{\partial\dot{C}_B^*}{\partial C_B^*} & \frac{\partial\dot{C}_B^*}{\partial C_A^*}\\ \frac{\partial\dot{C}_A^*}{\partial C_B^*} & \frac{\partial\dot{C}_A^*}{\partial C_A^*}\end{matrix}\right|_{SS} \qquad \ldots(23)$$

The eigen values (λs') of any of the biosystems can be determined by solving the following characteristic equation

$$\left|\tilde{J}-\lambda\tilde{I}\right|=0 \qquad \ldots(24)$$

According to Blanch and Clark[8], DiBiasio[10], Dutta *et al.*[11] and Xiu *et al.*[14], if any of the eigen values is positive then the steady state under consideration will be unstable in nature. On the other hand, the negative values of λs' indicate the stable nature of the steady

states under investigation. If the eigen value is a complex number, oscillation is observed around the steady state. The oscillation is of diverging or damped nature depending on positive and negative values of the real part of the eigen values respectively.

Global stability analysis

Phase plane analysis is one of the popularly used techniques for global/ general stability analysis of a system. By phase plane analysis the complete dynamic and steady state behaviour of a biochemical system is achieved. In the first stage of phase plane analysis the nondimensional cell mass balance equation has to be divided by that of substrate mass balance equation so that the incremental time $d\theta$ cancels out[8,10,11]. In the phase space (C^*_B, C^*_A) the phase trajectory can be defined by the oriented curves, generated by the movement of any point in it. The trajectory consisting of a single point corresponds to a rest point or stationary point in the space (C^*_B, C^*_A)[21]. The rest point represents the coordinates of the steady state corresponding to a particular set of values of system parameters, namely, dilution rate (D) and inlet substrate concentration ($C^*_{A,in}$).

For biodegradation of phenol the governing equation for phase plane analysis is as follows:

$$\frac{dC^*_B}{dC^*_A} = \frac{\left[\frac{\left(\delta^* C^{*2}_A + \gamma C^*_A\right)}{\left(K^*_S + C^*_A + \frac{C^{*2}_A}{K^*_I}\right)} - D^*\right] C^*_B}{D^*\left(C^*_{A,in} - C^*_A\right) - \frac{\left(\delta^* C^{*2}_A + \gamma C^*_A\right) C^*_B}{Y^*_{X/S}\left(K^*_S + C^*_A + \frac{C^{*2}_A}{K^*_I}\right)}} \quad \ldots(25)$$

The equations of line *A*, *B* & *C*, which subdivide the phase space (C^*_B, C^*_A) into six separatrices are as follows[8]:

$$\textbf{Line } \boldsymbol{A}\textbf{:}\ D^* = \frac{\left(\delta^* C^{*2}_A + \gamma C^*_A\right)}{\left(K^*_S + C^*_A + \frac{C^{*2}_A}{K^*_I}\right)} \quad \ldots(26)$$

$$\textbf{Line } \boldsymbol{B}\textbf{:}\ C^*_A = C^*_{A,in} - \frac{C^*_B}{Y^*_{X/S}} \quad \ldots(27)$$

$$\textbf{Line } \boldsymbol{C}\ D^*\left(C^*_{A,in} - C^*_A\right) = \frac{\left(\delta^* C^{*2}_A + \gamma C^*_A\right) C^*_B}{Y^*_{X/S}\left(K^*_S + C^*_A + \frac{C^{*2}_A}{K^*_I}\right)} \quad \ldots(28)$$

Apart from achieving the exact co-ordinates for the steady state point, the lines ***A***, ***B*** and ***C*** divide the phase plane into six separatrices. Initial points situated in a particular separatrix lead to similar and unique trajectories in approaching the rest/ steady state point for preset values of dilution rate and inlet substrate concentration. Thus, an overall characteristics regarding the locus of the steady state point as well as the trajectory patterns in the phase space (C^*_B, C^*_A) are obtained from the global stability analysis. If all the trajectories initiated from randomly chosen initial points in the entire phase space converge to the steady state point, the steady state is considered to be globally stable.

Response of system against step change

In this analysis, response of the biosystem obtained by deliberately introducing a step perturbation to the system parameters like dilution rate (D) and inlet substrate concentration ($C_{A,in}$) has been studied. The response pattern of concentrations of biomass and substrate in the time domain may be generated by the following analysis[22]:

On introduction of the perturbation variables (C^{*p}_A, C^{*p}_B, $C^{*p}_{A,in}$ and D^{*p}) in the Taylor series expansion of dimensionless variables C^*_A, C^*_B, $C^*_{A,in}$ and D^* in the model equations for the biosystem around their steady state (after truncation of higher order partial derivatives w.r.t. time) one gets,

$$\frac{dC^{*P}_A}{d\theta} = a_{11}C^{*P}_A + a_{12}C^{*P}_B + a_{13}C^{*P}_{A,in} + a_{14}D^{*P} \quad \ldots(29)$$

$$\frac{dC^{*P}_B}{d\theta} = a_{21}C^{*P}_A + a_{22}C^{*P}_B + a_{23}C^{*P}_{A,in} + a_{24}D^{*P} \quad \ldots(30)$$

The expressions of a_{11} to a_{24} are given in Table 2. Now the open loop transfer functions of this multivariable system may be determined considering $C^*_{A,in}$ and D^* as forcing functions. Mathematically, these can be expressed as follows:

$$C^{*P}_{A(S)} = G_{11}C^{*P}_{A,in(S)} + G_{12}D^{*P}_{(S)} \quad \ldots(31)$$

$$C^{*P}_{B(S)} = G_{21}C^{*P}_{A,in(S)} + G_{22}D^{*P}_{(S)} \quad \ldots(32)$$

where G_{11}, G_{12}, G_{21} and G_{22} are all transfer functions. Expressions of them are as follows:

$$G_{11} = \left[\frac{a_{13}\left(S - a_{22}\right) + a_{12}a_{23}}{\Psi}\right] \quad \ldots(33)$$

$$G_{12}=\left[\frac{a_{14}\left(S-a_{22}\right)+a_{12}a_{24}}{\psi}\right] \quad \ldots(34)$$

$$G_{21}=\left[\frac{a_{23}\left(S-a_{11}\right)+a_{13}a_{21}}{\psi}\right] \quad \ldots(35)$$

$$G_{22}=\left[\frac{a_{24}\left(S-a_{11}\right)+a_{14}a_{21}}{\psi}\right] \quad \ldots(36)$$

where,

$$\psi=S^2-\left(a_{11}+a_{22}\right)S+a_{11}a_{22}-a_{12}a_{21} \quad \ldots(37)$$

Applying fractional step inputs (σ), the concentration profiles of substrate and biomass in the time domain are obtained by inverse Laplace transformation of Eqs (31) and (32). The resulting equations are:

Table 2 — Expressions of coefficients used in Eqs (33)-(37)

Coefficients	Biodegradation of phenol
a_{11}	$-D_S^*-\dfrac{C_{BS}^*\left(2\delta^* K_S^* C_{AS}^*+\gamma K_S^*+\delta^* C_{AS}^{*2}-\dfrac{\gamma C_{AS}^{*2}}{K_I^*}\right)}{Y_{X/S}^*\left(K_S^*+C_{AS}^*+\dfrac{C_{AS}^{*2}}{K_I^*}\right)^2}$
a_{12}	$-\dfrac{\left(\delta^* C_{AS}^{*2}+\gamma C_{AS}^*\right)}{Y_{X/S}^*\left(K_S^*+C_{AS}^*+\dfrac{C_{AS}^{*2}}{K_I^*}\right)}$
a_{13}	D_S^*
a_{14}	$\left(C_{A,inS}^*-C_{AS}^*\right)$
a_{21}	$\dfrac{C_{BS}^*\left(2\delta^* K_S^* C_{AS}^*+\gamma K_S^*+\delta^* C_{AS}^{*2}-\dfrac{\gamma C_{AS}^{*2}}{K_I^*}\right)}{\left(K_S^*+C_{AS}^*+\dfrac{C_{AS}^{*2}}{K_I^*}\right)^2}$
a_{22}	$\dfrac{\left(\delta^* C_{AS}^{*2}+\gamma C_{AS}^*\right)}{\left(K_S^*+C_{AS}^*+\dfrac{C_{AS}^{*2}}{K_I^*}\right)}-D_S^*$
a_{23}	0
a_{24}	$-C_{BS}^*$

$$C_A^{*P}(\theta)=A_1+B_1e^{\xi\theta}\cos(\nu\theta)+\left(\frac{B_1\xi+C_1}{\nu}\right)e^{\xi\theta}\sin(\nu\theta) \quad \ldots(38)$$

$$C_B^{*P}(\theta)=A_2+B_2e^{\xi\theta}\cos(\nu\theta)+\left(\frac{B_2\xi+C_2}{\nu}\right)e^{\xi\theta}\sin(\nu\theta) \quad \ldots(39)$$

$$\xi=\frac{a_{11}+a_{22}}{2} \quad \ldots(40)$$

$$\nu=\sqrt{a_{11}a_{22}-a_{12}a_{21}-\xi^2} \quad \ldots(41)$$

A_1, A_2, B_1, B_2, C_1 and C_2 are constants whose expressions are given in Table 3.

Results and Discussion

For biodegradation of phenol it has already been observed by the previous investigators[17] that the system is guided by substrate inhibited kinetics of Haldane type. The kinetic parameters *viz*., specific growth rate (μ_m), saturation constant (K_S) and inhibition constant (K_I) have been determined by nonlinear regression analysis of the experimental data obtained in the present batch study. The values of the kinetic parameters have been enlisted in Table 1. Moreover the coefficients of the polynomial (c_1, c_2 and c_3) appeared in Eq. (3) have also been determined by nonlinear regression technique using the sets of experimental data taken at three different controlled *p*H (5, 6 and 7)[18]. The values of α_1, α_2, α_3, β_1, β_2 and β_3 are shown in Table 1.

Local stability analysis

Linear stability analysis has been used for the assessment of the local stability of a steady state. The steady state values at different operating conditions have been evaluated for phenol degradation system by solving Eqs (13)-(14) respectively. Eigen values, the indicator of stability of the steady states, have been found out by solving the characteristic equation

Table 3 — The expressions of A_1, A_2, B_1, B_2, C_1 and C_2 taking $C^*_{A,in}$ and D^* as forcing functions[11]

Forcing function	A_1	A_2	B_1	B_2	C_1	C_2
$C^*_{A,in}$	$\sigma(a_{12}a_{23}-a_{13}a_{22})/(a_{11}a_{22}-a_{12}a_{21})$	$\sigma(a_{13}a_{21}-a_{11}a_{23})/(a_{11}a_{22}-a_{12}a_{21})$	$-A_1$	$-A_2$	$\sigma(a_{11}a_{12}a_{23}-a_{12}a_{13}a_{21}+a_{12}a_{22}a_{23}-a_{13}a^2_{22})/(a_{11}a_{22}-a_{12}a_{21})$	$\sigma(a_{11}a_{13}a_{21}-a_{12}a^2_{21}a_{23}+a_{13}a_{21}a_{22}-a_{23}a^2_{11})/(a_{11}a_{22}-a_{12}a_{21})$
D^*	$\sigma(a_{12}a_{24}-a_{14}a_{22})/(a_{11}a_{22}-a_{12}a_{21})$	$\sigma(a_{14}a_{21}-a_{11}a_{24})/(a_{11}a_{22}-a_{12}a_{21})$	$-A_1$	$-A_2$	$\sigma(a_{11}a_{12}a_{24}-a_{12}a_{14}a_{21}+a_{12}a_{22}a_{24}-a_{14}a^2_{22})/(a_{11}a_{22}-a_{12}a_{21})$	$\sigma(a_{11}a_{14}a_{21}-a_{12}a^2_{21}a_{24}+a_{14}a_{21}a_{22}-a_{24}a^2_{11})/(a_{11}a_{22}-a_{12}a_{21})$

(Eq. 24) for different steady state values. The analysis has been carried out with the values of D^* in the range of 0.313-0.938 and those of $C^*_{A,in}$ in the range of 0.214-1. The eigen values and their corresponding steady states are given in Table 4. From the table it is evident that within the range of the variables studied, eigen values have negative real magnitudes indicating the occurrence of stable steady states.

Global stability analysis

In Fig. 2 the division of phase plane (C^*_B, C^*_A) into six separatrices (Separatrix I-VI) using Line *A*, *B* and *C* are shown for $D^* = 0.313$ and $C^*_{A,in} = 0.214$ for biodegradation of phenol. The rest point has coordinates (0.292, 0.0155). The characteristics behaviour of phase trajectories starting from each separatrix (starting point: P) and convergence to the rest point (end point: S) are shown in Fig. 3 for $D^* = 0.313$ and $C^*_{A,in} = 0.214$. This further indicates the stability of the steady state under the present operating conditions. From Fig. 3 it is seen that in Separatrix I, when $C^*_B(\theta)$ of the trajectory changes following overshoot feature, the change of $C^*_A(\theta)$of that trajectory can be characterized as underswing in nature during approaching to the rest point. Similarly in each separatrix (Separatrix I-VI), $C^*_B(\theta)$ and $C^*_B(\theta)$of each trajectory follows unique feature to reach the steady state. The nature of approach of trajectories from each separatrix are given in Table 5.

Table 4 — Eigen values for different operating conditions for the biodegradation of phenol using *Pseudomonas putida*

D^*	$C^*_{A,in}$	C^*_{BS}	C^*_{AS}	λ_1	λ_2
0.313	0.214	0.292	1.549×10^{-2}	−2.398	−5.944
0.313	0.357	0.457	1.549×10^{-2}	−3.784	−10.103
0.313	1.0	1.630	1.549×10^{-2}	−10.023	−28.820
0.625	0.214	0.267	3.221×10^{-2}	−2.579	−5.237
0.625	0.357	0.435	3.221×10^{-2}	−3.867	−9.101
0.625	1.0	1.603	3.221×10^{-2}	−9.663	−26.489
0.938	0.214	0.241	5.016×10^{-2}	−2.787	−4.609
0.938	0.357	0.411	5.016×10^{-2}	−3.988	−8.215
0.938	1.0	1.573	5.016×10^{-2}	−9.395	−24.435

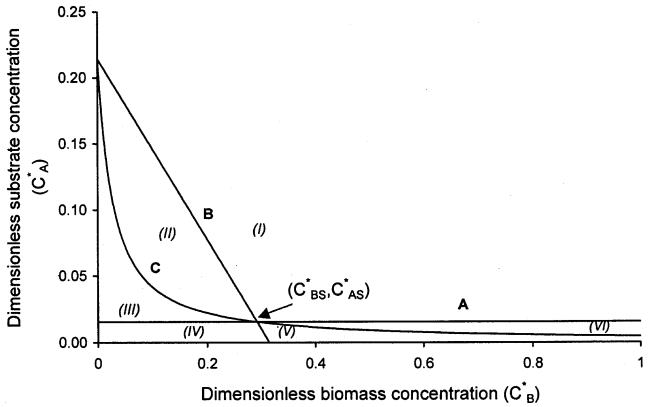

Fig. 2 — Classification of the transient response of (C^*_B,C^*_A) in each of the six separatrices in the (C^*_B,C^*_A) phase plane for biodegradation of phenol using *Pseudomonas putida* where $D^* = 0.313$, $C^*_{A,in} = 0.214$.

Response analysis

To investigate the system response to perturbation, the dynamic behaviour of the biosystem operating under different steady states has been studied for step perturbation of operating conditions of different amplitude. In Fig. 4 simulated transient response curves have been made by plotting dimensionless biomass concentration (C^*_B), calculated using Eq. (39), against dimensionless residence time (τ^*). The amplitude of step perturbation to $C^*_{A,in}$ has been used as a parameter and has been maintained at 1, 5, 10, 30, and 70% of the original value. The experimental results have been superimposed on the same set of curves in this figure. It is observed that the experimental data fit satisfactorily well with the simulated curves with sufficiently high index of correlation.

It is interesting to note that the response curves follow a pattern similar to control systems with

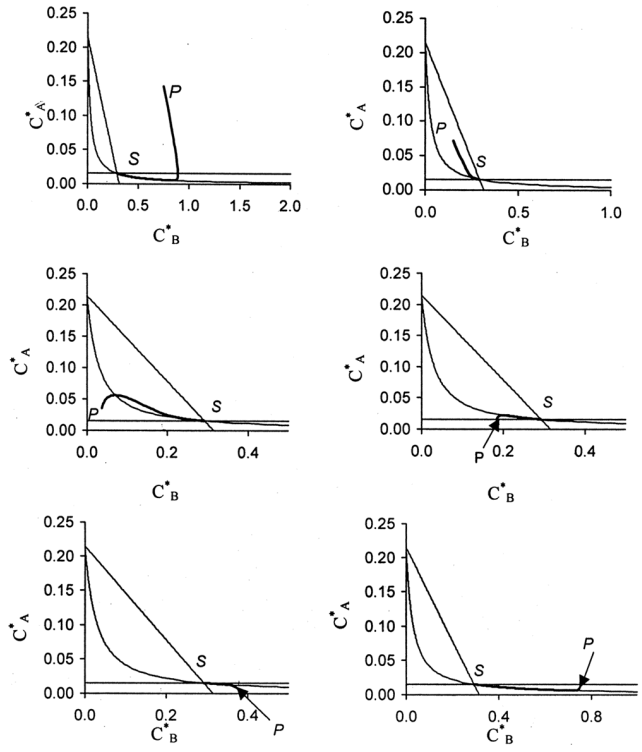

Fig. 3 — The transient response in each of the six separatrices. $D^* = 0.313$, $C^*_{A,in} = 0.214$.

Table 5 — Classification of the transient response of (C^*_B,C^*_A) in each of the six separatrices in the (C^*_B,C^*_A) phase plane for biodegradation of phenol using *Pseudomonas putida* where $D^* = 0.313$, $C^*_{A,in} = 0.214$

(C^*_{B0},C^*_{A0}) in separatrice	Behaviour of $C^*_B(\theta)$	Behaviour of $C^*_A(\theta)$
I	Overshoot	Underswing
II	Monotonic increase	Monotonic decrease
III	Monotonic increase	Overshoot
IV	Underswing	Overshoot
V	Monotonic decrease	Monotonic increase
VI	Monotonic decrease	Underswing

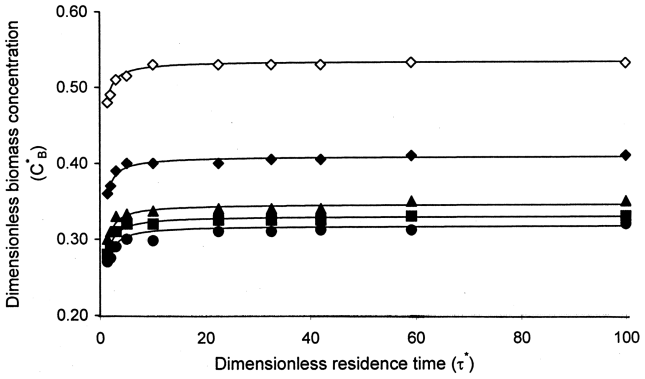

Fig. 4 — Simulated (——) and experimental ((●): σ = 1.0% $C^*_{A,in}$, (■): σ = 5.0% $C^*_{A,in}$, (▲): σ= 10.0% $C^*_{A,in}$, (♦): σ = 30.0% $C^*_{A,in}$, (◊): σ = 70.0% $C^*_{A,in}$) dynamic responses for dimensionless biomass concentration (C^*_B) against dimensionless residence time (τ^*) with fractional step change in $C^*_{A,in}$ (σ) as a parameter. In all cases $C^*_{A,in}$ = 0.060 for biodegradation of phenol.

second order lags. This is expected because the biosystem is having transfer function with second order lags (Eq. 35) obtained in case of C^*_B. The asymptotic value of dimensionless biomass concentration increases with the increase in the degree of amplitude of step change in $C_{A,in}$. On the other hand time required to attain the steady state is independent of the degree of the step change introduced in the system.

Conclusion

A comprehensive study of the stability analysis of the biodegradation of phenol has been done to identify the stable regime *a priori* to experimental runs with the values of D^* in the range of 0.313-0.938 and those of $C^*_{A,in}$ in the range of 0.214-1. Biodegradation of phenol follows substrate inhibited Haldane type kinetics. Two separate techniques, *viz.*, linear stability analysis and phase plane analysis have been used to study the characteristics of steady state from local and global points of view respectively. The phenol degrading biochemical system has been proved to be stable under the present operating conditions both from local and global perspectives. To study the dynamic response of a system to perturbation of any operating variable, the response analysis for the system has been performed for step perturbation of forcing variable, namely, inlet substrate concentration, of different amplitudes (1-70%).

It is evident from the foregoing discussion that the model equations developed to characterize the steady state condition of a biochemical system following substrate inhibited biochemical system can well explain the real time situation of the reactor behaviour. It is expected that these model equations will help in developing smart control strategy for bioreactor operation.

Nomenclature

C_A = Substrate concentration in outlet stream, g/dm^3

$C^*_A = \dfrac{C_A}{C_{A0,max}}$, Dimensionless substrate concentration in outlet stream

$\dot{C}^*_A = \dfrac{\partial C^*_A}{\partial \theta}$

C_{A0} = Initial substrate concentration in rector, g/dm^3

C_{A1} = Substrate concentration in exit stream from V_s at steady state as shown in Fig. 1, g/dm^3

$C_{A0,max}$ = Maximum initial concentration of substrate kept in the reactor, g/dm^3

$C^*_{A0} = \dfrac{C_{A0}}{C_{A0,max}}$, Dimensionless initial substrate concen-tration in reactor

$C_{A,in}$ = Substrate concentration in feed, g/dm^3

$C^*_{A,in} = \dfrac{C_{A,in}}{C_{A0,max}}$, Dimensionless substrate concentration in feed

$C_{A,inS}$ = Substrate concentration in feed corresponding to steady state, g/dm^3

$C^*_{A,inS} = \dfrac{C_{A,inS}}{C_{A0,max}}$, Dimensionless substrate concentration in feed at steady state

$C^{*P}_{A,in} = C^*_{A,in} - C^*_{A,inS}$

$C^{*P}_{A,in(S)}$ = Laplace transform of $C^{*P}_{A,in}$

C_{AS}= Steady state value of substrate concentration in outlet stream, g/dm^3

$C^*_{AS} = \dfrac{C_{AS}}{C_{A0,max}}$, Dimensionless steady state substrate concentration in outlet stream

$C^{*P}_A = C^*_A - C^*_{AS}$

$C^{*P}_{A(S)}$ = Laplace transform of C^{*P}_A

C_B = Biomass concentration in outlet stream, g/dm^3

C_{B0} = Initial concentration of biomass kept in the reactor, g/dm^3

$C_{B0,max}$ = Maximum initial concentration of biomass kept in the reactor, g/dm^3

$C_B^* = \frac{C_B}{C_{B0,max}}$

$C_{B0}^* = \frac{C_{B0}}{C_{B0,max}}$, Dimensionless initial biomass concentration in reactor

$\dot{C}_B^* = \frac{\partial C_B^*}{\partial \theta}$

C_{B1} = Biomass concentration in exit stream from V_s at steady state as shown in Fig. 1, g/dm^3

$C_{B,in}$ = Biomass concentration in feed, g/dm^3

C_{BS} = Steady state value of biomass concentration in outlet stream, g/dm^3

$C_{BS}^* = \frac{C_{BS}}{C_{B0,max}}$, Dimensionless steady state biomass concentration in outlet stream

$C_B^{*P} = C_B^* - C_{BS}^*$

$C_{B(S)}^{*P}$ = Laplace transform of C_B^{*P}

C_{T0} = Concentration of tracer in the inlet stream, g/dm^3

C_T = Concentration of tracer in the exit stream, g/dm^3

D= Dilution rate, ks^{-1}

D_S = Dilution rate corresponding to steady state, ks^{-1}

$D^* = \frac{D}{\mu_m}$, Dimensionless dilution rate

$D_S^* = \frac{D_S}{\mu_m}$, Dimensionless dilution rate corresponding to steady state

$D^{*P} = D^* - D_S^*$

$D_{(S)}^{*P}$ = Laplace transform of D^{*P}

$\tilde{I}$ = Unit vector

K_I = Inhibition constant, g/dm^3

$K_I^* = \frac{K_I}{C_{A0,max}}$

K_S = Saturation constant, g/dm^3

$K_S^* = \frac{K_S}{C_{A0,max}}$

S = Laplace domain variable

t = Time, ks

V = Reactor volume, dm^3

V_d = Dead zone as shown in Fig. 1, dm^3

$V_s = V - V_d$, Completely stirred volume of reactor as shown in Fig. 1, dm^3

v_0 = Total volumetric flow rate, dm^3/ ks

v_0 = Volumetric flow rate of the bypass flow as shown in Fig. 1, dm^3/ks

$v_s = v_0 - v_b$, dm^3/ks

$Y_{X/S}$ = Yield coefficient, g biomass/ g substrate

$Y_{X/S}^* = \frac{Y_{X/S} C_{A0,max}}{C_{B0,max}}$

Greek letters

α_1, α_2, α_3 = Coefficients used in Eqs (4)-(6), dm^3/g

β_1, β_2, β_3, = Coefficients used in Eqs (4)-(6)

$\delta^* = \delta C_{A0,max}$

η= Coefficient used in Eq. (10)

λ= Eigen value

μ= Specific growth rate, ks^{-1}

μ_m= Maximum specific growth rate, ks^{-1}

$\theta = t\mu_m$, Dimensionless time

ρ = Coefficient used in Eq. (10), g biomass/ g substrate

σ= Fractional step input (Table 3)

$\tau = \frac{V}{v_0}$

$\tau^* = \frac{\mu_m}{D}$, Dimensionless residence time

Superscripts

p = Perturbation variable

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