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Differential flow-induced chemical instability in a model enzyme mechanism

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General expressions are derived for the critical wave numbers for two- and three-species reaction mechanisms when their homogeneous steady states are destabilized by a relative flow of at least one species. Analysis of a model of the oscillating peroxidase-oxidase reaction indicates that the reaction exhibits this kind of instability. Computer simulations of the reaction-flow-diffusion equations generate traveling concentration waves under appropriate conditions.

I. INTRODUCTION

Recently, Rovinsky and Menzinger¹ reported that traveling concentration waves in the ferroin-catalyzed Belousov-Zhabotinsky (BZ) reaction are observed when ferroin is immobilized and the rest of the reagents flow through the ferroin. This is in excellent agreement with their theoretical prediction² based on a simple model of the BZ reaction. They referred to this phenomenon as "differential flow-induced chemical instability" (DIFICI). Similar to the role of diffusion in Turing pattern formation, a differential or relative flow spatially disengages the key species (e.g., the activator and inhibitor) resulting in a destabilization of the homogeneous steady state of the system.

In this paper, we derive general expressions for the bifurcation values of the parameters involved in DIFICI for two- and three-species models. In particular, the critical wave numbers are expressed as functions of the parameters. Our results are based on a linear stability analysis of the reaction-flow equations of the following form:

$$\frac{\partial X}{\partial t} = F(X, Y) - v \frac{\partial X}{\partial r}, \quad (1.1a)$$

$$\frac{\partial Y}{\partial t} = G(X, Y), \quad (1.1b)$$

where X and Y are concentrations, v is the flow velocity of species X relative to species Y . We assume v to be a parameter. For simplicity, we consider a one-dimensional space coordinate r . The functions F and G in Eq. (1.1) contain the kinetic terms due to the chemical reactions. It is of interest to identify the necessary conditions on the structure of a mechanism in order to exhibit DIFICI. For example, a two-species mechanism must have one species that is autocatalytic as already shown by Rovinsky and Menzinger.²

With an appropriate two-species mechanism, it has also been shown² that a critical wave number exists for any value of the flow velocity. However, adding a diffusion term ($D\partial^2 X/\partial r^2$) to the right-hand side of Eq. (1.1a) imposes a threshold value for v . In fact, the presence of the diffusion term drastically shrinks the region of instability in parameter space as will be demonstrated in the present work.

Finally, we show that the Alexandre-Dunford³ model of the oscillating peroxidase-oxidase reaction⁴ exhibits DIFICI. A situation is envisaged in which one of the species of the reaction is flowing relative to the enzyme and other species involved in the reaction. Using rate parameters predicted by stability analysis to generate DIFICI, computer simulations of the reaction-flow-diffusion equations of the model show traveling concentration waves. To the author's knowledge, this is the first illustration of DIFICI in a biochemical system.

II. LINEAR STABILITY ANALYSIS OF THE REACTION-FLOW-DIFFUSION EQUATIONS

A. Two-species mechanisms

We consider the reaction-flow Eqs. (1.1a) and (1.1b) and outline the steps in the derivation of the critical values of the parameters of "pure DIFICI," a phrase that we use to indicate the absence of diffusion. Let (X_0, Y_0) be the homogeneous steady state satisfying $F(X_0, Y_0) = G(X_0, Y_0) = 0$. Let x and y be the deviations from this steady state, i.e., $x = X - X_0$, $y = Y - Y_0$. We shall refer to the case $v = D = 0$ as the "homogeneous case," and variables referring to this case will contain a superscript zero. The linearized system of equations for the homogeneous case is

$$\frac{d}{dt} \begin{bmatrix} x \\ y \end{bmatrix} = \mathbf{M}_{(2)}^0 \begin{bmatrix} x \\ y \end{bmatrix}, \quad (2.1)$$

where $\mathbf{M}_{(2)}^0$ is the 2×2 Jacobian matrix with the components evaluated at the steady state

$$m_{11} = \frac{\partial F}{\partial X}, \quad m_{12} = \frac{\partial F}{\partial Y}, \quad m_{21} = \frac{\partial G}{\partial X}, \quad m_{22} = \frac{\partial G}{\partial Y}.$$

The eigenvalues, λ , of $\mathbf{M}_{(2)}^0$ are given by the roots of the characteristic polynomial

$$P^0(\lambda) = \det[\lambda \mathbf{I} - \mathbf{M}_{(2)}^0] = \lambda^2 + \alpha_1^0 \lambda + \alpha_2^0 = 0, \quad (2.2)$$

where \mathbf{I} is the identity matrix, and

$$\alpha_1^0 = -(m_{11} + m_{22})$$

and

$$\alpha_2^0 = m_{11}m_{22} - m_{21}m_{12}. \quad (2.3)$$

Local stability of (X_0, Y_0) requires that the eigenvalues have negative real parts which implies

$$\alpha_1^0 > 0$$

and

$$\alpha_2^0 > 0. \quad (2.4)$$

Let us now go back to the reaction-flow Eqs. (1.1a) and (1.1b) and linearize them about (X_0, Y_0) . A solution to the linearized equations is of the form $\begin{bmatrix} x \\ y \end{bmatrix} = \begin{bmatrix} c_1 \\ c_2 \end{bmatrix} e^{ikr} e^{\lambda t}$, where k is the wave number, λ is the eigenvalue, and c_1, c_2 are constants. The linearized reaction-flow equations are

$$\frac{\partial}{\partial t} \begin{bmatrix} x \\ y \end{bmatrix} = \mathbf{M}_{(2)} \begin{bmatrix} x \\ y \end{bmatrix}, \quad (2.5)$$

where

$$\mathbf{M}_{(2)} = \mathbf{M}_{(2)}^0 + \begin{bmatrix} -ivk & 0 \\ 0 & 0 \end{bmatrix}. \quad (2.6)$$

The eigenvalues of $\mathbf{M}_{(2)}$ are given by the characteristic polynomial

$$P(\lambda) = \det[\lambda \mathbf{I} - \mathbf{M}_{(2)}] = \lambda^2 + \alpha_1 \lambda + \alpha_2 = 0, \quad (2.7)$$

where

$$\alpha_1 = \alpha_1^0 + vki$$

and

$$\alpha_2 = \alpha_2^0 - m_{22} vki. \quad (2.8)$$

The eigenvalues λ are, in general, complex numbers of the form $(a + bi)$, where a and b are real numbers. An instability occurs when the real part of the eigenvalue, $\text{Re}(\lambda)$, changes sign, i.e., when $a = 0$. Let the critical eigenvalue be $\lambda_c = bi$. We substitute this into Eq. (2.7) and get

$$P(\lambda_c) = \text{Re } P + (\text{Im } P)i = 0, \quad (2.9)$$

where $\text{Re } P$ and $\text{Im } P$ are the real and imaginary parts of P , respectively, and are simultaneously zero to satisfy Eq. (2.9),

$$\text{Re } P = \alpha_2^0 - b_c^2 - b_c v k_c = 0, \quad (2.10)$$

$$\text{Im } P = b_c \alpha_1^0 - m_{22} v k_c = 0. \quad (2.11)$$

Eliminating b_c from Eqs. (2.10) and (2.11) gives

$$k_c = \frac{\alpha_1^0}{v} \sqrt{\frac{\alpha_2^0}{-m_{11} m_{22}}}. \quad (2.12)$$

This equation is identical to that already derived by Rovinsky and Menzinger.² This is also the equation that will give us the necessary conditions on the reaction terms if DIFICI has to arise. Since the numerator inside the radical sign is positive [see Eq. (2.4)], the denominator must also be positive, and thus m_{11} and m_{22} must have opposite signs. This implies that it is necessary that one, and only one, of the species must be autocatalytic. Equation (2.12) is identical to

$$k_c = \frac{-(m_{11} + m_{22})}{v} \sqrt{\frac{m_{12} m_{21}}{m_{11} m_{22}}} - 1. \quad (2.13)$$

From this equation we can now summarize the necessary and sufficient conditions for DIFICI in two-species mechanisms described in Eqs. (1.1a) and (1.1b) with $v > 0$,

$$(m_{11} + m_{22}) < 0$$

and

$$m_{12} m_{21} < m_{11} m_{22} < 0. \quad (2.14)$$

These inequalities ultimately determine the appropriate values for the rate parameters.

When a diffusion term for the flowing species is included in Eq. (1.1a), the linearized matrix $\mathbf{M}_{(2)}$ in Eq. (2.6) becomes

$$\mathbf{M}_{(2)}' = \mathbf{M}_{(2)}^0 + \begin{bmatrix} -vki - Dk^2 & 0 \\ 0 & 0 \end{bmatrix} \quad (2.15)$$

and the corresponding expression for the critical wave number is no longer as explicit as Eq. (2.12); instead, k_c is now obtained from the roots of the following polynomial (which is cubic in k_c^2):

$$c_6 k_c^6 + c_4 k_c^4 + c_2 k_c^2 + c_0 = 0, \quad (2.16)$$

where

$$c_6 = m_{22} D^3,$$

$$c_4 = 2m_{22} D^2 \alpha_1^0 - \alpha_2^0 D^2 + m_{22} v^2 D,$$

$$c_2 = -2\alpha_2^0 \alpha_1^0 D + m_{22}^2 v^2 + m_{22} D (\alpha_1^0)^2 + m_{22} v^2 \alpha_1^0,$$

$$c_0 = -\alpha_2^0 (\alpha_1^0)^2.$$

Note that Eq. (2.16) reduces to Eq. (2.12) when $D = 0$.

B. Three-species mechanisms

We consider the following set of reaction-flow equations and perform a linear stability analysis similar to the two-species case:

$$\begin{aligned} \frac{\partial X}{\partial t} &= F(X, Y, Z) - v \frac{\partial X}{\partial r}, \\ \frac{\partial Y}{\partial t} &= G(X, Y, Z), \\ \frac{\partial Z}{\partial t} &= H(X, Y, Z). \end{aligned} \quad (2.17)$$

Let (X_0, Y_0, Z_0) be a steady state of the homogeneous case, and let $\mathbf{M}_{(3)}^0$ be the 3×3 Jacobian matrix associated with the linearized system of equations. Linear stability of (X_0, Y_0, Z_0) requires the Hurwitz determinants, Δ_i ($i = 1, 2, 3$), to be positive,⁵

$$\Delta_1 = \alpha_1^0 > 0, \quad (2.18a)$$

$$\Delta_2 = \alpha_1^0 \alpha_2^0 - \alpha_3^0 > 0, \quad (2.18b)$$

$$\Delta_3 = \alpha_3^0 \Delta_2 > 0 \text{ (implying } \alpha_3^0 > 0), \quad (2.18c)$$

where the α_i^0 's are the coefficients of the characteristic polynomial

$$P^0(\lambda) = \det[\lambda \mathbf{I} - \mathbf{M}_{(3)}^0] = \lambda^3 + \alpha_1^0 \lambda^2 + \alpha_2^0 \lambda + \alpha_3^0 = 0 \quad (2.19)$$

and

$$\alpha_1^0 = -(m_{11} + m_{22} + m_{33}), \quad (2.20a)$$

$$\alpha_2^0 = (m_{11}m_{22} - m_{21}m_{12}) + (m_{22}m_{33} - m_{23}m_{32}) + (m_{11}m_{33} - m_{13}m_{31}), \quad (2.20b)$$

$$\alpha_3^0 = -\det \mathbf{M}_{(3)}^0. \quad (2.20c)$$

The eigenvalues of the linearized matrix $\mathbf{M}_{(3)}$ associated with the reaction-flow Eqs. (2.17) are given by the characteristic polynomial

$$P(\lambda) = \det[\lambda \mathbf{I} - \mathbf{M}_{(3)}] = \lambda^3 + \alpha_1 \lambda^2 + \alpha_2 \lambda + \alpha_3 = 0, \quad (2.21)$$

where

$$\alpha_1 = \alpha_1^0 + vki, \quad (2.22a)$$

$$\alpha_2 = \alpha_2^0 - (m_{22} + m_{33})vki, \quad (2.22b)$$

$$\alpha_3 = \alpha_3^0 - (m_{23}m_{32} - m_{22}m_{33})vki. \quad (2.22c)$$

Substituting the critical eigenvalue $\lambda_c = b_c i$ into Eq. (2.21) gives an equation similar to Eq. (2.9) with

$$\text{Re } P = \alpha_3^0 - b_c^2 \alpha_1^0 + b_c (m_{22} + m_{33})vk_c = 0, \quad (2.23)$$

$$\text{Im } P = b_c \alpha_2^0 - (m_{23}m_{32} - m_{22}m_{33} + b_c^2)vk_c - b_c^3 = 0. \quad (2.24)$$

From Eq. (2.23), we get the critical value for k_c ,

$$k_c = \frac{b_c^2 \alpha_1^0 - \alpha_3^0}{vb_c(m_{22} + m_{33})}. \quad (2.25)$$

Substituting Eq. (2.25) into Eq. (2.24) gives a fourth-order polynomial in b_c ,

$$\Phi(b_c) = d_4 b_c^4 + d_2 b_c^2 + d_0 = 0. \quad (2.26)$$

$\Phi(b_c)$ is actually quadratic in b_c^2 and can be solved exactly. The exact expressions for the coefficients d_4 , d_2 , and d_0 are given in Appendix A. These coefficients are functions of the rate parameters.

Equation (2.25) provides the following boundary of DIFICI (where $k_c = 0$):

$$\alpha_1^0 b_c^2 - \alpha_3^0 = 0. \quad (2.27)$$

This equation reminds us of the boundary of instability for the homogeneous case where the second Hurwitz determinant vanishes [see Eq. (2.18b)]. Indeed we find that setting $b_c^2 = \alpha_2^0$ would satisfy Eq. (2.26). This result tells us that DIFICI emanates from the boundary of the homogeneous instability where $\Delta_2 = 0$. An illustration of this is given in Fig. 3(b).

When the diffusion term for the flowing species is included in Eq. (2.17), the exact expressions for the critical wave number becomes very unwieldy and is not reported here. The numerical calculations in the next section will

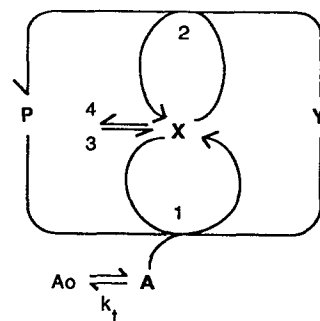


FIG. 1. Network diagram of the Alexandre-Dunford (AD) model of the peroxidase-oxidase reaction.

show that the critical values for pure DIFICI [given by Eq. (2.25)] provide necessary conditions for instability when diffusion is included [see Fig. 3(a)].

III. APPLICATION TO A MODEL OF THE PEROXIDASE-OXIDASE (PO) REACTION

The following model was proposed by Alexandre and Dunford (AD) (Ref. 3) for the oscillating PO reaction:⁴



A network diagram is given in Fig. 1 which clearly illustrates the structure of the mechanism. Species B is assumed to have a constant concentration so that the rate of reaction (3.3) is constant and equal to $k_3 = k_{3i}B$. A_0 denotes a constant reservoir of A . For simplicity of notation, we use the same symbols for the species and their corresponding concentrations. There is one conservation condition for the total enzyme concentration E_t , namely,

$$P + Y = E_t \text{ (constant)}; \quad (3.6)$$

E_t is determined by the initial conditions for P and Y . We used the following three independent kinetic equations in the analysis:

$$dA/dt = -k_1 PAX + k_t(A_0 - A), \quad (3.7)$$

$$dX/dt = k_1 PAX + k_2 XY + k_3 - k_4 X, \quad (3.8)$$

$$dY/dt = k_1 PAX - k_2 XY, \quad (3.9)$$

where $P = P(Y)$ is given in Eq. (3.6). The components of the linearized matrix, $\mathbf{M}_{(3)}^0$, for the homogeneous case are given in Appendix B. The steady states (A_0, X_0, Y_0) were determined explicitly as functions of the parameters in Ref. 3.

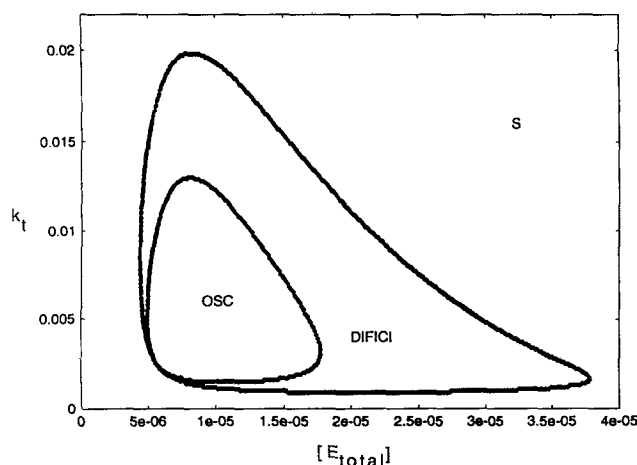


FIG. 2. Phase diagram of the AD model on the k_t - E_t parameter plane. OSC=homogeneous oscillations; DIFICI=pure DIFICI region for νk_c fixed at 0.1; S=stable region. Parameter values, $k_1=2.0 \times 10^{10}$, $k_2=5.0 \times 10^5$, $k_3=1.0 \times 10^{-10}$, $k_4=1.25$, $A_0=1.5 \times 10^{-5}$.

Alexandre and Dunford³ suggested the following correspondence of the model species and those identified in the actual reaction $A=O_2$, P =ferriperoxidase (the native enzyme), Y =oxyperoxidase, and X =NAD \cdot radical, the autocatalytic species in the mechanism. We now assume that we can design an experiment in which species A (oxygen) is flowing relative to the other species. This seems to be quite feasible because techniques of immobilizing the peroxidase enzyme, as well as binding NADH to certain enzymes are available.⁶

In order to demonstrate how the presence of the flow term ($-\nu \partial A / \partial r$) enlarges the unstable region, we show in Fig. 2 the oscillatory region for the homogeneous case and the pure DIFICI region for a fixed value of νk_c . Note that according to Eq. (2.25), for the pure DIFICI case, the critical values of ν and k_c are not independent; the product νk_c is determined by the kinetic parameters. It is shown that, indeed, the DIFICI region extends well beyond the homogeneous oscillatory region.

Figure 3 demonstrates how the presence of the diffusion term ($D \partial^2 A / \partial r^2$) changes the unstable region in parameter space. The boundaries between the stable and unstable regions were determined by a linear stability analysis of the reaction-diffusion-flow equations. In Fig. 3(a), to the right of the diagonal line ($D=0$) is the unstable region representing pure DIFICI; it shows that there is a critical wave number for any ν . When diffusion of the flowing species is considered, the unstable region is drastically reduced as exemplified by the regions enclosed by the curves labeled $D=1.0 \times 10^{-3}$ and 0.5×10^{-3} . These curves show that diffusion imposes a maximum wave number above which DIFICI is impossible; furthermore, there is a minimum flow velocity below which no DIFICI is possible. This figure shows that as D approaches zero the DIFICI region widens and it is clear that this region will be identical to that of the right side of the diagonal line when $D=0$. It is also important to note that the pure DIFICI line ($D=0$) serves as a left boundary for any unstable

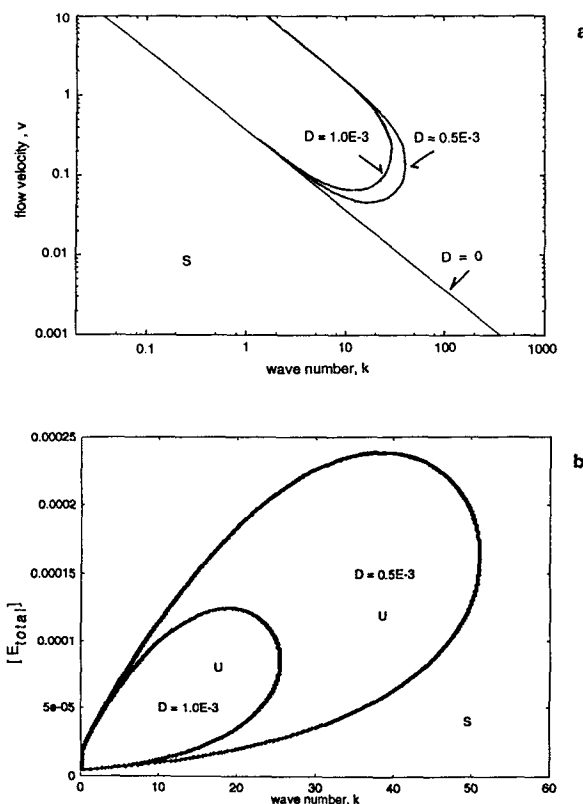


FIG. 3. (a) Bifurcation diagram for the flow velocity vs wave number for the AD model at three different values of the diffusion coefficient D . S=stable region. (b) Bifurcation diagram for the total enzyme concentration vs wave number for two different values of D . S=stable; U=unstable. The values of all other parameters are the same as in Fig. 2.

region that includes nonzero values of D . Thus, in practice, one first calculates the ($D=0$)-line and then searches for the unstable region to the right of this line for systems with nonzero D values.

Another bifurcation diagram involving the total enzyme concentration is shown in Fig. 3(b). The unstable regions (labeled U) increases when D decreases. This diagram also shows that for a fixed D , there is a maximum total enzyme concentration above which no mode is unstable. There is also a minimum $[E_t]$ level below which the homogeneous steady state is always stable. Note that this minimum occurs at zero wave number; at $k_c=0$, the narrow range of $[E_t]$ which gives instability represents the levels of the enzyme that generate homogeneous oscillations in the system.

IV. COMPUTER SIMULATIONS

DIFICI leads to traveling concentration waves as verified by integrating the reaction-flow-diffusion equations of the AD model. For the fixed values of $[E_t]=6.0 \times 10^{-5}$ and $D=0.001$, the bifurcation diagram in Fig. 3 predicts that wave numbers between 4.2 and 24.2 are unstable (these correspond to wavelengths of ~ 1.5 and 0.26 distance units, respectively). For the simple purpose of showing the result of the instability, the reaction system is sim-

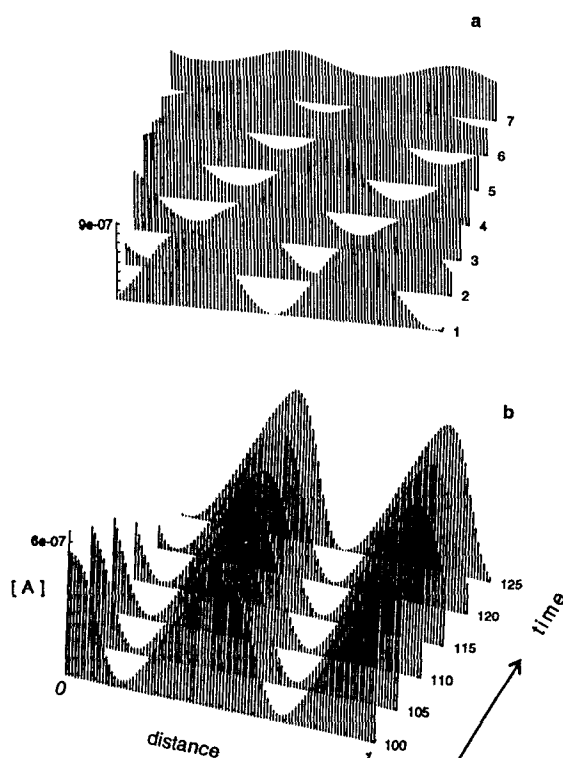


FIG. 4. (a) Concentration waves of species A travel with the flow at an early stage of DIFICI. (b) These waves eventually travel in the direction opposite the flow. Parameter values are $E_t = 6.0 \times 10^{-5}$, $D = 0.001$, the rest are the same as in Fig. 2. Periodic boundary conditions are used.

ulated in a closed ring (one-dimensional) so that periodic boundary conditions can be used in the integration of the partial differential equations. Initial concentrations of species X and Y are chosen to be those of the homogeneous steady state. The initial inhomogeneous perturbation was introduced via a sinusoidal concentration profile of species A (specifically, a sine wave with wave number of 4π). The transient dynamics [shown in Fig. 4(a)] exhibits concentration waves that travel in the same direction as the flow. These initial waves are very similar to the initial perturbation because, at this stage, the kinetics due to the chemical reactions is still insignificant (note that if the reaction terms are set to zero in the reaction-flow-diffusion equations, any initial perturbation will be propagated at the same velocity as the flow; the presence of diffusion merely flattens the waves and does not affect their velocity). The concentration waves of species A are then observed to slow down, and after ~ 15 time units they slowly start to travel in the direction opposite to the flow (in fact, between 15 and 22 time units, the waves are fairly stationary). Figure 4(b) shows the long-time behavior of the system. Waves with fairly constant amplitudes persist and travel in the direction opposite to the flow. The spatial variation of the concentrations of species X and Y were also checked and found to exhibit concentration waves traveling in the same direction as those of species A . The details of the interac-

tion among the nonlinear reaction kinetics, the flow, and the geometry of the reaction vessel is not completely understood at this time.

V. CONCLUDING REMARKS

We have presented an example of a biochemical mechanism that exhibits an instability induced by a differential flow between species involved in the reaction. After deriving exact expressions for the critical wave numbers in terms of the parameters of the reaction kinetics, we have established the conditions for DIFICI. Equations (2.12) and (2.25) provide the exact expressions for the bifurcation points of DIFICI in 2- and 3-species mechanisms.

The peroxidase-oxidase (PO) reaction exhibits a wide range of nonlinear dynamical behavior including bistability, sustained oscillations, and chaos.⁴ We propose that the Alexandre-Dunford mechanism is a valid abstraction of the detailed PO mechanism⁴ for the following reasons: (a) the required presence of an enzyme feedback loop is satisfied; (b) as in detailed mechanisms, two loops involving the autocatalytic species X are present and both loops interact with the enzyme feedback loop; and (c) the phase diagram shown in Fig. 2 agrees qualitatively with the diagram generated from detailed mechanisms.⁴ The results of this paper offer the prediction that the peroxidase-oxidase reaction can exhibit traveling concentration waves under appropriate experimental conditions.

ACKNOWLEDGMENTS

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APPENDIX A

Coefficients of the polynomial $\Phi(b_c)$ given in Eq. (2.26),

$$d_4 = m_{11},$$

$$d_2 = (m_{22}m_{33} - m_{23}m_{32})\alpha_1^0 + (m_{22} + m_{33})\alpha_2^0 + \alpha_3^0,$$

$$d_0 = (m_{23}m_{32} - m_{22}m_{33})\alpha_3^0.$$

APPENDIX B

Components of the Jacobian matrix for the Alexandre-Dunford model,

$$m_{11} = -k_t - k_1 X_0 (E_t - Y_0),$$

$$m_{12} = -k_1 A_0 (E_t - Y_0),$$

$$m_{13} = k_1 A_0 X_0,$$

$$m_{21} = k_1 X_0 (E_t - Y_0),$$

$$m_{22} = k_1 A_0 (E_t - Y_0) + k_2 Y_0 - k_4,$$

$$m_{23} = -k_1 A_0 X_0 + k_2 X_0,$$

$$m_{31} = k_1 X_0 (E_t - Y_0),$$

$$m_{32} = k_1 A_0 (E_t - Y_0) - k_2 Y_0,$$

$$m_{33} = -k_1 A_0 X_0 - k_2 X_0.$$

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