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Elimination of Oscillations in Fermentation Processes

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Fermentation processes that are used to produce ethanol exhibit oscillatory behavior and for periods of time during these oscillations, ethanol production decreases substantially and there is considerable loss of residual substrates. There has been a considerable amount of work demonstrating oscillatory behavior in fermentation processes. The aim of this work is to demonstrate simple strategies to eliminate the oscillatory behavior in fermentation processes involving both Zymomonas mobilis *and* Saccharomyces cerevisiae. *The oscillatory behavior is caused by the existence of Hopf bifurcations and it is demonstrated that very minor changes in the input conditions can eliminate the Hopf bifurcation points. In the case of* Zymomonas mobilis*, it is shown that the addition of a small amount of substrate and/or the key component of the biomass and/or product in the input stream causes the disappearance of the Hopf bifurcation points while in* Saccharomyces cerevisiae *fermentation process, a very minor increase in the input oxygen supply produces the same result. The aim of this work is not only to demonstrate the existence of the Hopf bifurcations in the fermentation problems, but also to provide strategies to eliminate them.* © 2010 American Institute of Chemical Engineers *AIChE J,* 57: 2397–2405, 2011

Keywords: fermentation, bifurcation, oscillation, ethanol, biomass

Introduction

The field of chemical engineering affords a tremendous amount of research possibilities to applied mathematicians. One of the main reasons for this is the existence of oscillations and multiple steady-states in complex chemical processes that are caused by the interactions between the transport, thermodynamic and the kinetic phenomena. In addition, biochemical engineering, the importance of which has grown rapidly during recent years, involves biological phenomena which adds substantially to the already existing complexity. Fermentation processes involving both *Zymomonas mobilis* and *Saccharomyces cerevisiae* have demonstrated the existence of oscillatory behavior which are caused by the existence of Hopf bifurcations. This article deals with the demonstration of simple strategies to eliminate the oscillation causing Hopf bifurcation points. While there are several articles that demonstrate the existence of oscillations and the Hopf bifurcation points that cause them to occur, this article provides techniques to avoid/eliminate them because of their wasteful nature. In the case of the *Zymomonas mobilis* fermentation process, it is demonstrated that the addition of a small amount of substrate and/or the key component and/or product in the input stream results in a the disappearance of the Hopf bifurcations, while in the fermentation process involving *Saccharomyces cerevisiae*, it is shown that the addition of a small amount of oxygen in the input stream has the similar desired effect.

Background

Ethanol can be produced by fermentation from raw materials that contain carbohydrates. The raw materials that are used to produce ethanol can be divided into three categories;

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(a) sugars, (b) starch, and (c) cellulose materials. Sugar can be converted to ethanol directly, whereas starch must be hydrolyzed first and cellulose must be converted to sugars by the use of mineral acids.[1] Once the sugars are formed, enzymes from yeast or bacteria can readily ferment them to ethanol. Traditionally, ethanol is produced in fermentation with yeast strains such as *Saccharomyces cerevisiae*, which under anaerobic conditions, metabolizes glucose to produce ethanol.

Many bacteria such as *Zymomonas mobilis*[2] can be used to produce ethanol from glucose[3,4] showed that the bacteria, *Zymomonas mobilis*, can cause much higher yield than traditional yeasts.

Sustained or damped oscillations of biomass, substrate and product (ethanol) concentrations have been observed by several workers in continuous cultures of the bacteria *Zymomonas*[5–9] and of the yeast *Saccharomyces* at temperatures above 303 K.[10–12] Oscillatory behavior is linked directly to strong product inhibition.[7,9,11] linked the oscillatory behavior with the additive effect of substrate excess and product inhibitions.

Perego et al.[10,11] clearly demonstrate that for *Saccharomyces* and *Clostridium*, oscillations take place at high values of feed substrate concentrations while these oscillations seem to disappear for low values of the feed substrate concentrations. This same trend was demonstrated for *Zymomonas mobilis*[5–7,13] Experiments performed by Bruce et al.,[9] observed strong damping of oscillations when substrate concentrations fell to small amounts. Ghommidh et al.,[8] proposed a tricompartment model which for substrate saturation portrays sustained oscillations in a continuous culture of *Zymomonas*. However, this model fails to demonstrate stable behavior when there is substrate limitation. This issue was remedied by Jarzebski[14] who modified the tri-compartment model to better portray the dynamics of the fermentation process.

The existence of oscillations, periodic cycles, regions of multiplicity and chaotic behavior in *Zymomonas* fermentation process problems have been demonstrated in refs. [15–18]. The fermentation of *Saccharomyces cerevisiae* has also demonstrated the existence of sustained oscillations as shown in refs. [19–21]. Jones and Komapla[22] model which is commonly used to describe the fermentation of *Saccharomyces cerevisiae* has computationally demonstrated oscillatory behavior as shown by Zhang and Henson.[23] This oscillatory behavior was analyzed in detail by Simpson et al.[24]

While there is a lot of work demonstrating the existence of these undesirable oscillations and the Hopf bifurcation points that cause them, there is clearly a need to develop strategies to eliminate them. This article demonstrates simple strategies to eliminate the oscillations for both the *Zymomonas mobilis* and the *Saccharomyces cerevisiae* fermentation process problems.

The article is organized as follows: First the model equations for both the *Zymomonas mobilis* and the *Saccharomyces cerevisiae* fermentation processes are presented. Then, the numerical technique used for the calculations is briefly described. The strategies to eliminate the Hopf bifurcation points for both the fermentation problems are then discussed. Examples demonstrating the effectiveness of the strategies are then presented followed by the conclusions.

Model equations

In this section, the model equations for both the *Zymomonas mobilis* and the *Saccharomyces cerevisiae* fermentation processes are presented. The model suggested by Garhyan and Elnashaie[17] is used for the *Zymomonas mobilis* fermentation process while the model proposed by Jones and Kompala[22] is used for the *Saccharomyces cerevisiae* fermentation process.

Zymomonas *fermentation model*

The fermentation model suggested by Jobses et al.,[7,17,18] describes the fermentation of *Zymomonas mobilis*. Here the rate of formation expression for the key compound e is given by

$$r_{\mathrm{e}} = f(C_{\mathrm{S}}) f(C_{\mathrm{P}})(C_{\mathrm{e}}) \tag{1}$$

The formation rate of the key component (which is an RNA in the biomass) is a function of the substrate concentration, is inhibited by ethanol and its activity is expressed in terms of concentration only.[7] *S* represents the substrate, while *P* represents the product. $f(C_{\mathrm{S}})$ is given by the Monod type equation

$$f(C_{\mathrm{S}}) = \left(\frac{C_{\mathrm{s}}}{K_{\mathrm{S}} + C_{\mathrm{s}}}\right) \tag{2}$$

The function $f(C_{\mathrm{P}})$ is given by a polynomial (Jobses et al., 1986)

$$f(C_{\mathrm{P}}) = k_1 - k_2 C_{\mathrm{P}} + k_3 C_{\mathrm{P}}^2 \tag{3}$$

The dynamic model for the four components, substrate (*S*), microorganism or biomass (*X*), the key compound (*e*), and product (*P*) are given by the following equations.

$$\frac{dC_{\mathrm{e}}}{dt} = [k_1 - k_2 C_{\mathrm{P}} + k_3 C_{\mathrm{P}}^2]\left(\frac{C_{\mathrm{S}} C_{\mathrm{e}}}{K_{\mathrm{S}} + C_{\mathrm{S}}}\right) + D_{\mathrm{in}} C_{\mathrm{e0}} - D_{\mathrm{out}} C_{\mathrm{e}} \tag{4}$$

$$\frac{dC_{\mathrm{X}}}{dt} = P\left(\frac{C_{\mathrm{S}} C_{\mathrm{e}}}{K_{\mathrm{S}} + C_{\mathrm{S}}}\right) + D_{\mathrm{in}} C_{\mathrm{X0}} - D_{\mathrm{out}} C_{\mathrm{X}} \tag{5}$$

$$\frac{dC_{\mathrm{S}}}{dt} = \mathrm{P}\left(\frac{-1}{Y_{\mathrm{SX}}}\right)\left(\frac{C_{\mathrm{S}} C_{\mathrm{e}}}{K_{\mathrm{S}} + C_{\mathrm{S}}}\right) - m_{\mathrm{S}} C_{\mathrm{X}} + D_{\mathrm{in}} C_{\mathrm{e0}} - D_{\mathrm{out}} C_{\mathrm{e}} \tag{6}$$

$$\frac{dC_{\mathrm{P}}}{dt} = \mathrm{P}\left(\frac{1}{Y_{\mathrm{PX}}}\right)\left(\frac{C_{\mathrm{S}} C_{\mathrm{e}}}{K_{\mathrm{S}} + C_{\mathrm{S}}}\right) + m_{\mathrm{P}} C_{\mathrm{X}} + D_{\mathrm{in}} C_{\mathrm{P0}} - D_{\mathrm{out}} C_{\mathrm{P}} - \left(\frac{a}{V_{\mathrm{f}}}\right)(C_{\mathrm{P}} - C_{\mathrm{PM}}) \tag{7}$$

The last term in Eq. 7, is the term corresponding to the removal of ethanol by a membrane and this term is zero for the Jobses et al.[7] model. This additional term was incorporated in refs. [17,18] who also considered the membrane side of the equations which are given by

Table 1. Base Set of Parameters Used for the *Zymomonas Mobilis* Fermentation(Garhyan and Elnashaie[17]).

Parameter	Value
K_1	16.0
K_2	0.497
K_3	0.00383
m_s	2.16
m_p	1.1
Y_{sx}	0.02444498
Y_{px}	0.0526315
K_s	0.5
P	0.1283
$D_{M\ in}$	0.5
C_{x0}	0
C_{P0}	0
C_{e0}	0
V_F	0.003
V_M	0
ρ	789

$$\frac{dC_{PM}}{dt} = (\frac{a}{V_M})(C_P - C_{PM}) + D_{M,in}C_{PM0} - D_{M,out}C_{PM} \tag{8}$$

where

$$D_{M,out} = D_{M,in} + \frac{a(C_P - C_{PM})}{V_M(\rho)} \tag{9}$$

$$D_{out} = D_{in} - \frac{a(C_P - C_{PM})}{V_M(\rho)} \tag{10}$$

and

$$a = A_M P \tag{11}$$

D represents the dilution rate, Y_{SX} and Y_{PX} the yield factors of biomass on substrate and product, and, the k's are empirical constants, V is the volume, A_M the area of permeation and P the permeability of the membrane. m_S and m_P are maintenance factors based on substrate requirements and product formation. The subscripts M and F represent the membrane side and the fermentation side. Table 1 gives the base values of the variables and constants used in the *Zymomonas* fermentation problem.

Saccharomyces cerevisiae *model*

Jones and Kompala[22] have developed a detailed cybernetic model to represent the *Saccharomyces cerevisiae* fermentation process. Along three available pathways r_i the cybernetic variables u_i and v_i represent the optimal strategies for enzyme synthesis and activity. The variables u_i and v_i are given by the equations

$$u_i = \frac{r_i}{\sum_j r_j} \tag{12}$$

$$v_i = \frac{r_i}{\max_j r_j} \tag{13}$$

while the expressions for the pathways r_i are given by

$$r_1 = \mu_1 e_1 \frac{G}{K_1 + G} \tag{14}$$

$$r_2 = \mu_2 e_2 (\frac{E}{K_2 + E})(\frac{O}{K_{O_2} + O}) \tag{15}$$

$$r_3 = \mu_3 e_3 (\frac{G}{K_3 + G})(\frac{O}{K_{O_3} + O}) \tag{16}$$

With these growth rate equations, the balance equations[22] are given by

$$\frac{dX}{dt} = X \sum_i r_i v_i - DX \tag{17}$$

$$\frac{dG}{dt} = (G_0 - G)D - (\frac{r_1 v_1}{Y_1} - \frac{r_2 v_2}{Y_2})X - \phi_4 (C\frac{dX}{dt} + X\frac{dc}{dt}) \tag{18}$$

$$\frac{dE}{dt} = -DE + (\phi_1 \frac{r_1 v_1}{Y_1} - \frac{r_2 v_2}{Y_2})X \tag{19}$$

$$\frac{dO}{dt} = k_L a(O^* - O) - (\phi_2 \frac{r_2 v_2}{Y_2} + \phi_3 \frac{r_3 v_3}{Y_3})X \tag{20}$$

$$\frac{de_i}{dt} = \alpha u_i \frac{S_i}{K_i + S_i} - (\sum_j r_j v_j + \beta) e_i + \alpha^* \tag{21}$$

$$\frac{dC}{dt} = \gamma_3 r_3 v_3 - (\gamma_1 r_1 v_1 + \gamma_2 r_2 v_2)C - \sum_i (r_i v_i)C \tag{22}$$

G, E, and O represent the concentrations of glucose, ethanol and dissolved oxygen. μ_i represents the modified growth rate constant. K_i and K_{Oi} represent the saturation constants for the carbon substrate and the dissolved oxygen for each metabolic pathway. G_0 represents the inlet glucose feed concentration, X is the cell mass concentration and $k_L\ a$ represents the dissolved oxygen mass transfer coefficient. Y is the yield coefficient, while α and β represent the enzyme synthesis and decay rate constants. The stoichiometric coefficients for the intercellular storage carbohydrate synthesis and consumption are given by ϕ_i and γ_i. Table 2 gives the base values of the variables and constants used.

Table 2. Base Set of Parameters Used for the *Saccharomyces cerevisiae* Fermentation Problem[22].

Parameter	Value
G_0	10 gm/l
Y_1, Y_2, Y_3	(0.16, 0.75, 0.6) gg^{-1}
ϕ_1, ϕ_2, ϕ_3, ϕ_4	0.403, 2, 1, 0.95
O^*	7.5 mg/l
α	0.3
α^*	0.1
β	0.7
K_1, K_2, K_3	0.05, 0.01, 0.001
K_{O_2}	0.01 mg/l
K_{O_3}	2.2 mg/l
γ_i (i = 1,2,3)	10, 10, 0.8
$\mu_{i,\max}$ (i = 1,2,3)	0.44, 0.19, 0.36

Table 3. Changes in the Input Conditions that Cause the Disappearance of the Hopf Bifurcations in the *Zymomonas mobilis* Fermentation Problem.

Problem Number (Figure Numbers in Brackets)	Bifurcation Paramater	Original Values of Variables Before Modification Which Give Hopf Bifurcation Points	Modified Values of Variables that Causes Disappearance of Hopf Bifurcation Points
1 (1 and 3)	D_{in}	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.02$
		$C_{\mathrm{x0}} = 0$	$C_{\mathrm{x0}} = 0.02$
		$C_{\mathrm{p0}} = 0$	$C_{\mathrm{p0}} = 0.02$
2 (2 and 4)	D_{in}	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.01$
3 (5 and 6)	C_{s0}	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.01$
		$C_{\mathrm{x0}} = 0$	$C_{\mathrm{x0}} = 1.0$
		$C_{\mathrm{p0}} = 0$	$C_{\mathrm{p0}} = 0.1$
4 (7 and 8)	A_{M}	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.175$
		$C_{\mathrm{x0}} = 0$	$C_{\mathrm{xo}} = 0.9$
5 (9 and 10)	A_{M}	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.2$
		$C_{\mathrm{x0}} = 0$	$C_{\mathrm{x0}} = 0.8$
6 (11 and 12)	$D_{\mathrm{M,in}}$	$C_{\mathrm{e0}} = 0$	$C_{\mathrm{e0}} = 0.06$
		$C_{\mathrm{x0}} = 0$	$C_{\mathrm{x0}} = 1.08$

Numerical technique used to locate the singularities

The program CL_MATCONT[25,26] was used to locate singularities in the set of ODE described in the previous section. For the resulting equilibrium curve $f(u,\alpha) = 0$, where α is the continuation parameter, and u the remaining variables, the defining function is $F(x) = f(u,\alpha) = 0$ where $x = (u,\alpha) \in R^{n+1}$ and v is the tangent vector at x. Three test functions will be defined as

$$\phi_1 = \det\begin{pmatrix} F_{\mathrm{x}} \\ v^T \end{pmatrix} \tag{23}$$

$$\phi_2 = \det(2f_{\mathrm{u}}(u,\alpha) * I_{\mathrm{n}}) \tag{24}$$

$$\phi_3(u,\alpha) = v_{\mathrm{n+1}} \tag{25}$$

For the existence of a branch point, $\phi_1 = 0$, while ϕ_2 and ϕ_3 are zero for a Hopf bifurcation point and a limit point. * indicates the bialternate product.

Results and Discussion

Different strategies are used to eliminate the oscillation causing Hopf bifurcations in the case of both *Zymomonas mobilis* and the *Saccharomyces cerevisiae* fermentation processes. In the case of the *Zymomonas mobilis* fermentation process it is shown that the addition of a small amount of substrate and/or product and/or the internal key component of the biomass, in the input stream causes the elimination of the Hopf bifurcation points. On the other hand, in the case of the *Saccharomyces cerevisiae* fermentation process it is demonstrated that a minor increase in the oxygen content in the input air stream causes the Hopf bifurcation points to disappear. Several examples for both the fermentation process problems will be presented in the next section.

Elimination of the Hopf bifurcation points in Zymomonas mobilis *fermentation*

In this section a description of the problems for the *Zymomonas mobilis* fermentation process is provided. Table 3 gives a description of the values of the variables (original and modified) that cause the Hopf bifurcation points to disappear.

D_{in} *is bifurcation parameter (Cases 1 and 2)*

A Hopf bifurcation point was found for a $C_{\mathrm{s0}} = 150.3$ at a D_{in} value of 0.052152 and the concentrationvalues at this Hopf bifurcation point were $C_{\mathrm{e}} = 0.076791$, $C_{\mathrm{x}} = 1.449101$, $C_{\mathrm{s}} = 31.013036$, and $C_{\mathrm{p}} = 58.097959$. A similar Hopf bifurcation point was found for a $C_{\mathrm{s0}} = 200$ at a D_{in} value of 0.053626 and the concentrationvalues at this Hopf bifurcation point were $C_{\mathrm{e}} = 0.079314$, $C_{\mathrm{x}} = 1.469907$, $C_{\mathrm{s}} = 80.674792$, and $C_{\mathrm{p}} = 58.079387$. The D_{in} values for these 2 Hopf bifurcations are the same as those reported by Garhyan and Elnashaie,[17] and this confirms that the program developed is correct. These Hopf bifurcation points are shown in Figures 1 and 2.

For the problem where the C_{S0} value is 150.3 we increased C_{e0}, C_{x0}, C_{p0} values of 0.02 each we have three neutral saddles and a limit point for the C_{e},C_{x},C_{s},C_{p} and D_{in} values of (0.047283, 1.356092, 6.106871, 71.008319, 0.032711), (0.150153, 1.970166, 2.098558, 71.926608, 0.062180), (0.170114, 2.018045, 1.624551, 72.080441, 0.065103), and (0.196452, 2.046838, 1.102573, 72.293609, 0.066685). Similarly, for a C_{S0} value of 200 and C_{e0} values

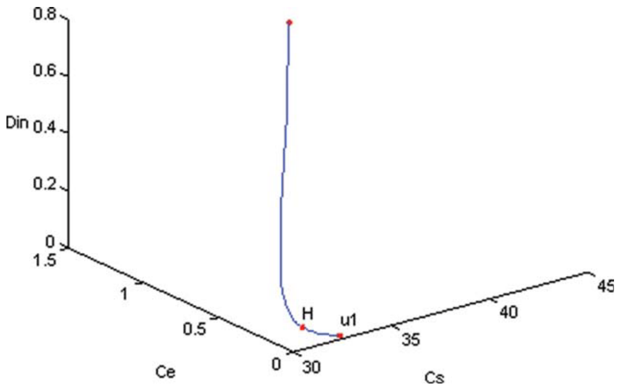

Figure 1. Hopf bifurcationin case 1.

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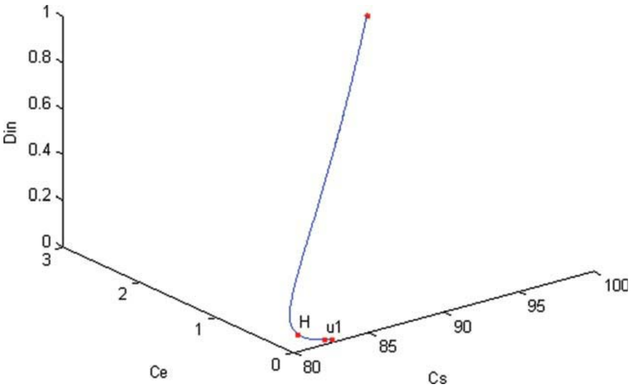

Figure 2. Hopf bifurcationin case 2.

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of 0.01 we have three neutral saddles and a limit point for the C_e,C_x,C_s,C_p and D_{in} values (0.105093, 1.855506, 52.674916, 71.633423, 0.056106), (11.003571, 4.635427, 5.830798, 90.405466, 2.186318), (11.041867, 4.637539, 5.751172, 90.442155, 2.190533), and (12.178437, 4.696030, 3.454274, 91.504906, 2.265430). This is demonstrated in Figures 3 and 4. Neutral saddles are not bifurcation points and do not cause oscillatory behavior.

C_{s0}*is the bifurcation parameter (Case 3)*

Using C_{s0}as a bifurcation parameter and a D_{in} value of 0.045 (Garhyan and Elnashaie, 2004), a Hopf bifurcation point was found at a C_{s0} value of 131.299635. The C_e,C_x,C_s,C_p values at this point are (0.062779, 1.340037, 12.170129, 58.217222). For this same problem (using C_{s0} as a bifurcation parameter,and a D_{in} value of 0.045) and $C_{x0}C_{p0}$ C_{e0} values of 1.0, 0.1, and 0.01, three neutral saddles and a limit point were found at C_e,C_x,C_s,C_p, and C_{s0} values of (0.051644, 2.078164, 7.758828, 71.384721, 151.607768), (0.059601, 2.081473, 2.225302, 71.528482, 146.368418), (0.066196, 2.084149, 1.401146, 71.644737, 145.782155), and (0.075150, 2.087692, 0.933959, 71.798641, 145.629901). This is shown in Figures 5 and 6.

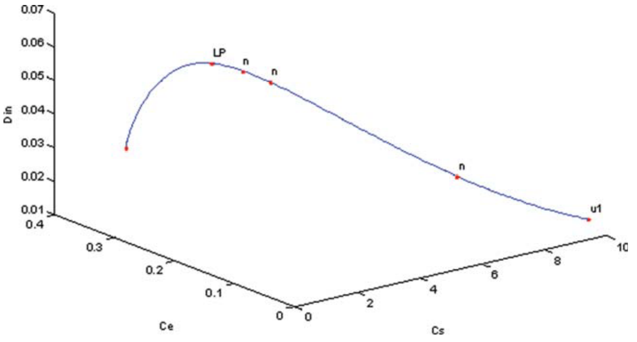

Figure 3. Elimination of Hopf bifurcation in case 1.

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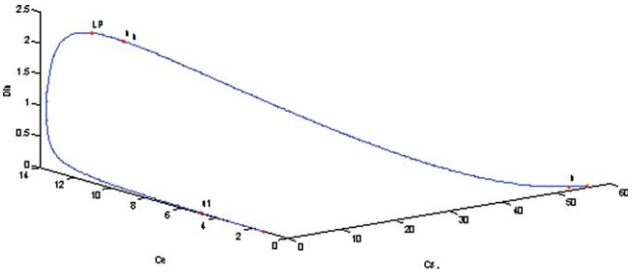

Figure 4. Elimination of Hopf Bifurcation in case 2.

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A_M *as a bifurcation parameter*

Case 4. Using A_M as a bifurcation parameter we have found a Hopf bifurcation point at C_e, C_x,C_s,C_p $C_{PM}A_M$ value of (0.064356, 1.481497, 15.061500, 58.284328, 7.249975, 0.000168). The C_{s0} value was 150.3 and the D_{in} value was 0.0424.[17] For this same problem (using A_M as a bifurcation parameter) we have found two limit points at C_e,C_x,C_s,C_p $C_{PM}A_M$ values of (0.047620, 1.962382, 7.524886, 65.490020, 4.899631, 0.000095) and (0.060014, 2.024450, 1.933278, 67.103367, 5.923243, 0.000114). The $C_{s0}C_{X0}C_{e0}$ and D_{in} values are150.3, 0.9, 0.175, and 0.0424. This can be seen in Figures 7 and 8.

Case 5. Using A_M as a bifurcation parameter[17] and (C_{s0} = 200; D_{in} = 0.04584 and the $D_{M,in}$ = 0.5 [Botero et al.[18]]) we have found a Hopf bifurcation point at C_e, C_x, C_s, C_p $C_{PM}A_M$ values of (0.073800, 1.619666, 59.315330, 58.242414, 10.679704, 0.000266). For this same problem when the $C_{s0}C_{X0}C_{e0}$ D_{in} and $D_{M,in}$ values are 200, 0.8, 0.2, and 0.04584 and 0.5 and using A_M as a bifurcation parameter we have found three neutral saddles and one limit point at C_e, C_x, C_s, C_p $C_{PM}A_M$ values of (0.057516, 2.061102, 52.572648, 66.340911, 6.665108, 0.000132), (0.066795, 2.279953, 34.573369, 67.659469, 14.094758, 0.000313), (0.091051, 2.648803, 3.925244, 68.803506, 27.092918, 0.000786) and (0.054944, 2.002003, 57.516056, 65.342005, 5.223639, 0.000102). This can be seen in Figures 9 and 10.

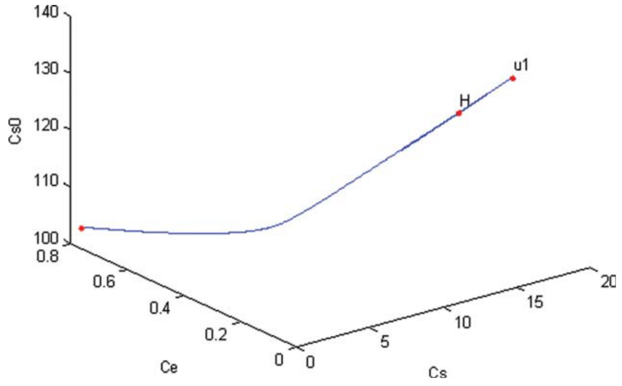

Figure 5. Hopf bifurcationin case 3.

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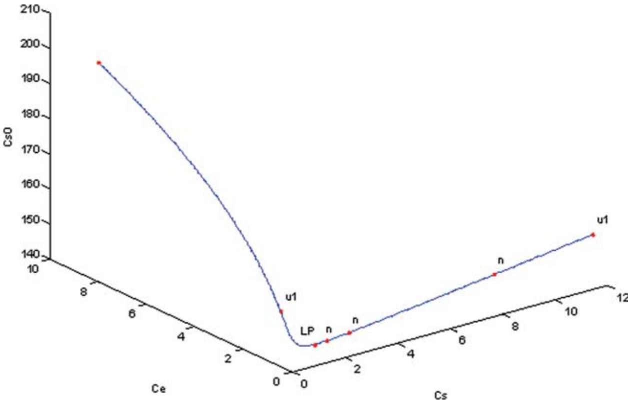

Figure 6. Elimination of Hopf Bifurcation in case 3.

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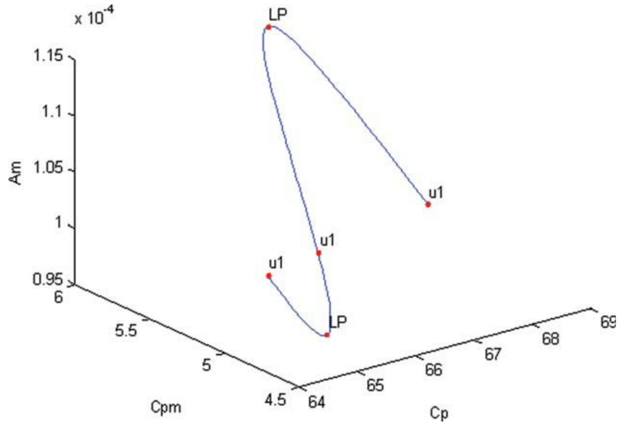

Figure 8. Elimination of Hopf bifurcation in case 4.

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$D_{M,in}$ *is the bifurcation parameter (Case 6)*

Using $D_{\mathrm{M,in}}$ as a bifurcation parameter[18] we have found a Hopf bifurcation point at C_{e}, C_{x}, C_{s}, C_{p} $C_{\mathrm{PM}}D_{\mathrm{M,in}}$ values of (0.080235, 1.771958, 46.831227, 58.248013, 58.168622, 0.135165). The C_{s0} value was 200, the A_{M}value was 0.25 and the D_{in} value was 0.04584. For this problem, when the C_{s0} value was 200, the A_{M}value was 0.25 and the D_{in} value was 0.04584 and increasing the C_{X0} and C_{e0} values are 1.08 and 0.06, using $D_{\mathrm{M,in}}$ as a bifurcation parameter we have found two neutral saddle points and a limit point at C_{e}, C_{x}, C_{s}, C_{p} $C_{\mathrm{PM}}D_{\mathrm{M,in}}$ values of (0.089523, 2.794614, 2.539933, 70.919035, 70.793055, 0.173192); (0.097551, 2.804331, 1.716270, 71.019143, 70.891783, 0.174821); and (0.107515, 2.810127, 1.215240, 71.141906, 71.013990, 0.175253). The C_{s0} value was 200, the A_{M}value was 0.25 and the D_{in} value was 0.4584. The C_{X0} and C_{e0} values are 1.08 and 0.06. This can be seen in Figures 11 and 12.

Table 3 summarizes all the results in this section. It is shown that in all the cases described, where Hopf bifurcations were present, addition of a small amount of the internal key component and/or substrate and/or product in the input stream causes the Hopf bifurcation points to disappear and limit points to appear. This is very significant because it provides a very easy way to replace the unwanted oscillations with more acceptable steady-state branches. While all the work so far deals with the demonstrations of oscillations this work shows an easy strategy for eliminating them.

Elimination of the Hopf bifurcation points in Saccharomyces cerevisiae *fermentation*

In this section, examples of *Saccharomyces cerevisiae* fermentation problems where the Hopf bifurcation points disappear when the value of O^* was modified slightly are presented. The original and the modified values of O^* are presented in Table 4. In all the cases D is the bifurcation parameter. The original value of O^* in all the cases was 7.5 which is the concentration of the oxygen in air. In all the cases studied it was seen that the addition of a small amount of extra oxygen results in the disappearance of the Hopf bifurcation.

Case 7

The first *Saccharomyces cerevisiae* problem we study is the problem discussed in Ref. 24. In this case the value of $k_{\mathrm{L}}\ a$ is 150 and the G_0 value is 10. When the O^* value is

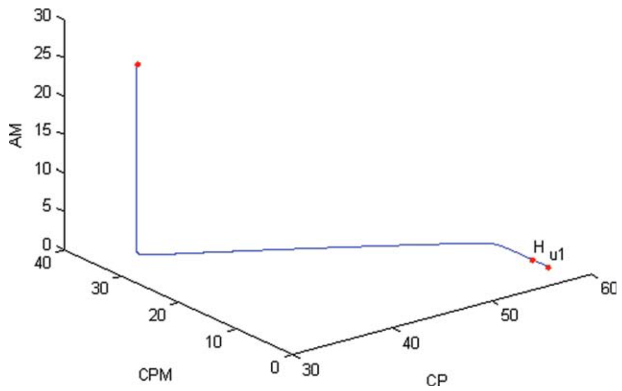

Figure 7. Hopf Bifurcation in case 4.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

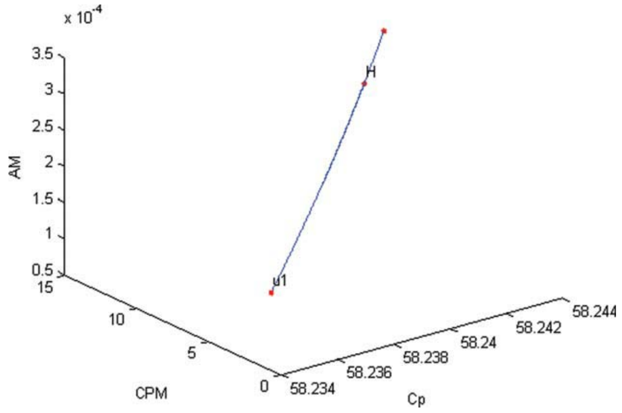

Figure 9. Hopf Bifurcationin case 5.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

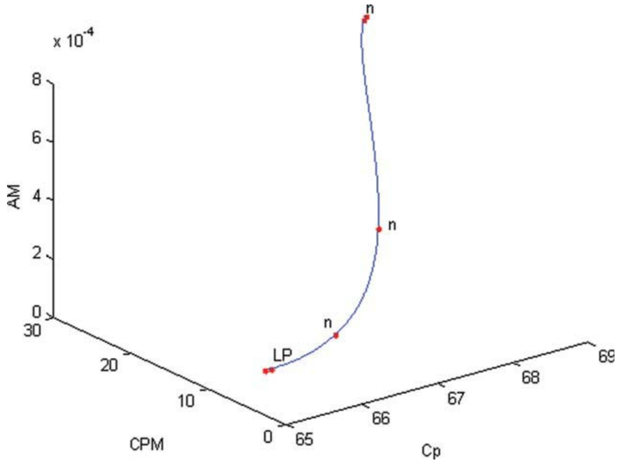

Figure 10. Elimination of Hopf bifurcation in case 5.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

7.5 mg/l there are 2 Hopf points, 5 neutral saddles and one limit point. This is indicated in Figure 13. Changing O^* from 7.5 mg/l to 9.5 mg/l results in the disappearance of the Hopf bifurcation points. Three of the neutral saddles and the limit point remain. This is shown in Figure 14

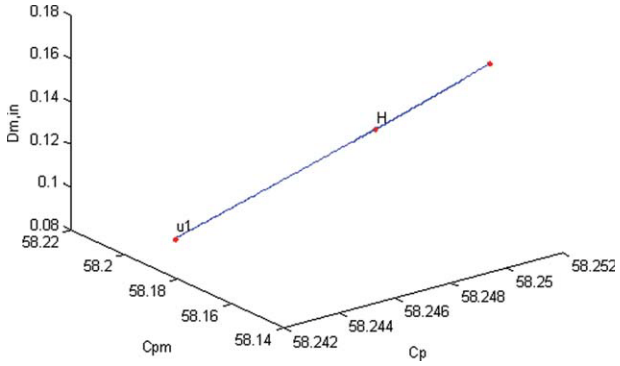

Figure 11. Hopf bifurcation in case 6.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

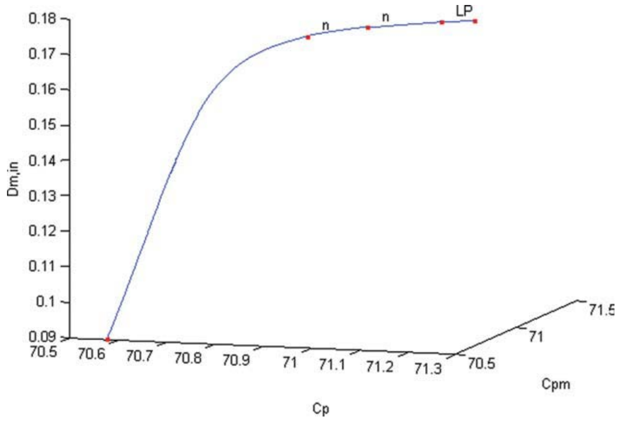

Figure 12. Elimination of Hopf Bifurcation in case 6.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 4. Changes in the Input Condition that Cause the Disappearance of the Hopf Bifurcations in the *Saccharomyces cerevisiae* Fermentation Problem

Problem Number (Figure Numbers in Brackets)	Original Value of O^* Before Modification Which give Hopf Bifurcation Points	Modified Values of O^*that Causes Disappearance of Hopf Bifurcation Points
1 (13 and 14)	7.5	9.5
2 (15 and 16)	7.5	8.0
3 (17 and 18)	7.5	8.6

Case 8

In the second *Saccharomyces cerevisiae* example,[23] for a $k_L\ a$ value of 225, a G_0 value of 11.5, and an O^* value is

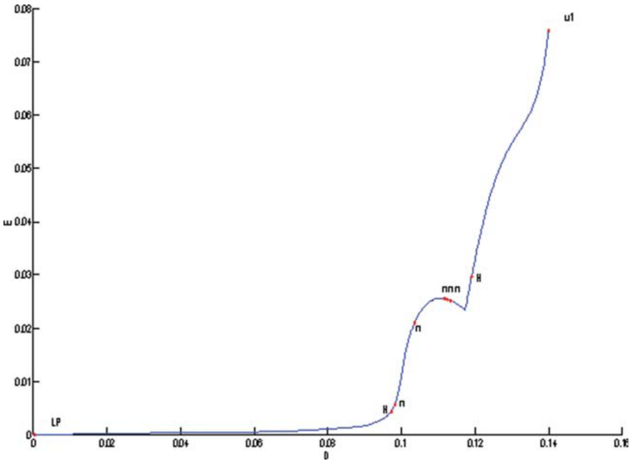

Figure 13. Hopf Bifurcation in case 7.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

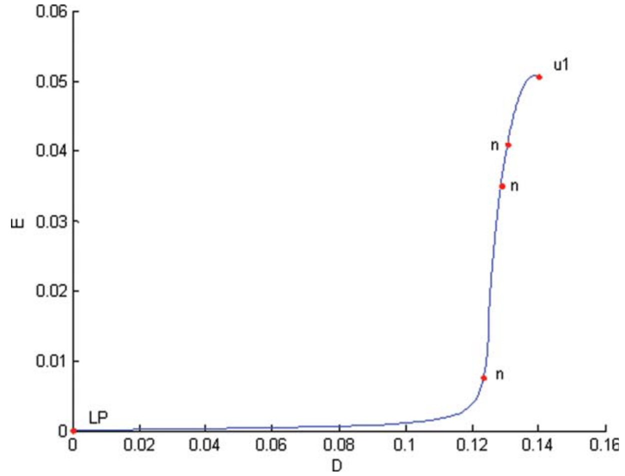

Figure 14. Elimination of Hopf Bifurcation in case 7.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

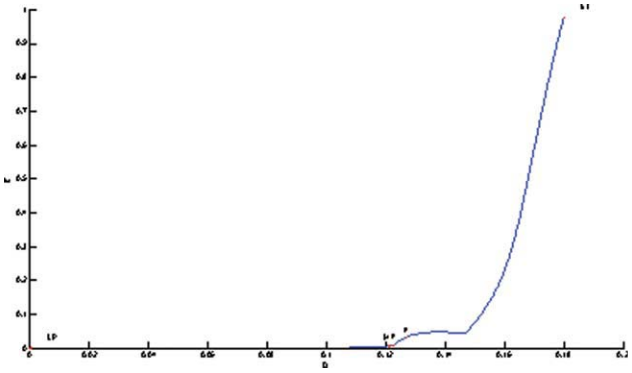

Figure 15. Hopf Bifurcation in case 8.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

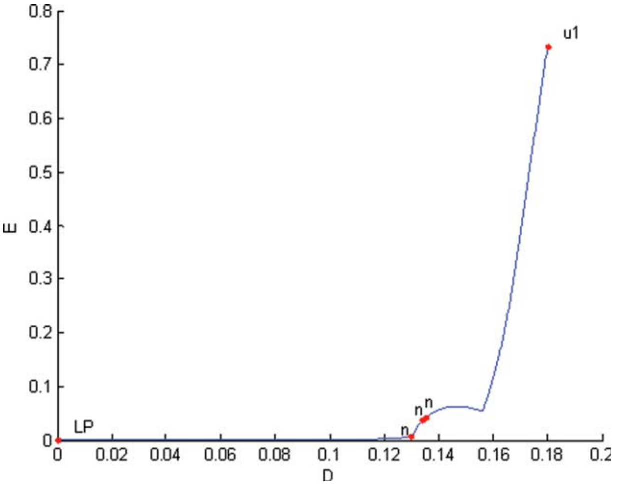

Figure 16. Elimination of Hopf Bifurcation in case 8.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

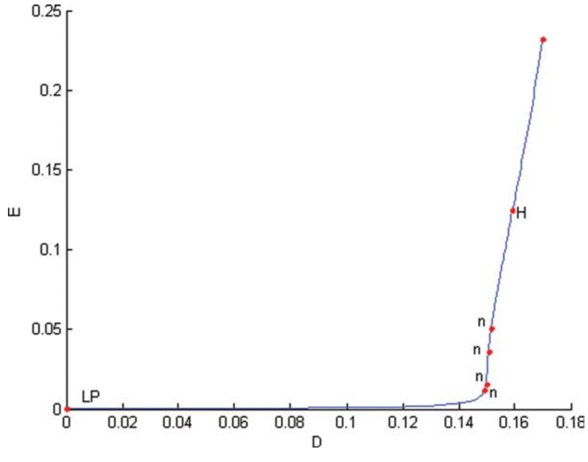

Figure 17. Hopf Bifurcation in case 9.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

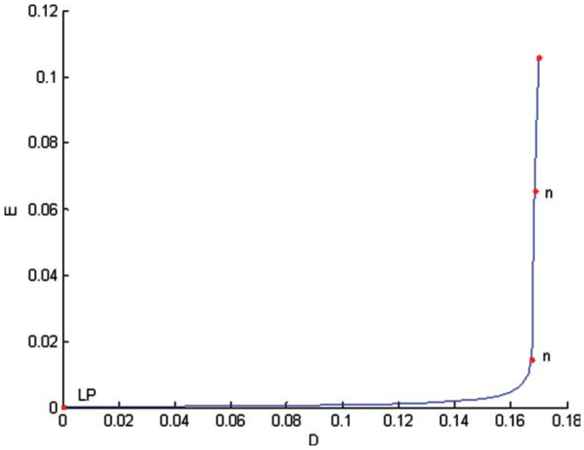

Figure 18. Elimination of Hopf Bifurcation in case 9.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

7.5 mg/l, we get a Hopf bifurcation point, two neutral saddles and a limit point. This is shown in Figure 15. When the O^* value is changed to 8.0 mg/l, the Hopf bifurcation point disappears. Three neutral saddles and a limit point are observed. This is shown in Figure 16.

Case 9

In the third *Saccharomyces cerevisiae* example, For a $k_L\ a$ value of 225, a G_0 value of 8.75, and an O^* value is 7.5 mg/l, we get one Hopf bifurcation point, four neutral saddles and a limit point. This is shown in Figure 17. Increasing the O^* value is 8.6 mg/l, results in two neutral saddles and one limit point as shown in Figure 18.

In all the *Saccharomyces cerevisiae* cases, it is shown that increasing the input oxygen concentration by a small amount removes the oscillation causing Hopf bifurcations. This summarized in Table 4.

Conclusions

While there is considerable amount of literature demonstrating numerically and graphically, the existence of the Hopf bifurcations points that cause the oscillations in the fermentation process involving both *Zymomomonas mobilis* and *Saccharomyces cerevisiae* this article provides very easy to implement strategies to eliminate them. It is also shown in the case of *Zymomomonas mobilis* fermentation process the Hopf bifurcations disappear when small amounts of internal key component/and/orsubstrate and/orproduct are added to the input stream. In the case of the *Saccharomyces cerevisiae* fermentation process, it is demonstrated that a small increase in the oxygen supply to the fermentation process would result in the elimination of the unwanted oscillation causing Hopf bifurcations. Apart from the different kinetics the most significant difference between the two fermentations is the fact that the *Saccharomyces cerevisiae* is aerobic (oxygen flow can be manipulated)and the *Zymomomonas mobilis* fermentation is anaerobic. This work therefore provides easy techniques for removing the unwanted and wasteful oscillatory behavior that occur in both these fermentations.

Acknowledgments

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Notation

$C =$ concentration
$D =$ dilution rate
$V =$ volume
$E =$ biomass for *Zymomonas mobilis*, ethanol for *Saccharomyces cerevisiae*
$X =$ cell mass concentration in *Saccharomyces cerevisiae* fermentation problem
$O =$ oxygen concentration
$O^* =$ oxygen solubility limit (normal value 7.5 is when air is fed in K_1, K_2 K_3 empirical constants
$P =$ permeability
$A =$ area
$Y =$ yield factor

Subscripts

e = key component of biomas
0 = input
P = product
M = membrane
S = substrate
X = biomass

Literature Cited

1. Senn T, Pieper HJ. *Biotechnology*, Vol. 6: *Ethanol-Classical methods*. Weinheim, Germany: Wiley-VCH, 1996:62–66.
2. Baratti JC, Bu'lock JD. *Zymomonas mobilis*, a bacterium for ethanol production. *Biotechnol Adv*. 1986;4:95–115.
3. Gunasekharan P, Chandra Raj K. Ethanol fermentation technology-*Zymomonas mobilis*. *Curr Sci*. 1999;77:56–58.
4. Picataggio SK, Zhang M. *Biocatalyst development for bio-ethanol production from hydrolysates*. In: Wyman CE, editor. *Handbook on bioethanol, Production and Utilization*. Washington, DC: Taylor and Francis, 1996.
5. Lee KJ, Tribe DE, Rogers PL. Ethanol production by *Zymomonas mobilis* in continuous culture at high glucose concentrations. *Biotechnol Lett*. 1979;1:421–426.
6. Lee KJ, Skotnicki ML, Tribe DE, Rogers PL. Kinetic Studies on a highly productive strain of *Zymomonas mobilis*. *Biotechnol Lett*. 1980;2:339–344.
7. Jobses I, Egbertsa ML, GTC, Luyben KCAM, Roels JA. Fermentation kinetics of *Zymomonas mobilis* at high ethanol concentrations; Oscillations in continuous cultures. *Biotechnol Bioeng*. 1986;28:868.
8. Ghommidh C, Vaija J, Bolarinwa S, Navarro JM. Oscillatory behavior of *Zymomonas mobilis* in continuous cultures, a simple stochastic model, *Biotechnol Lett*. 1989;2:659–664.
9. Bruce LJ, Axford DB, Ciszek B, Daugulis JA. Extractive fermentation by *Zymomonas mobilis* and the control of oscillatory behavior. *Biotechnol Lett*. 1991;13:292–296.
10. Perego L, Cabral JM, Dias S, Koshimizu LH, De Melo Cruz MR, Borzani W, Vairo MLR. Influence of temperature, dilution rate and sugar concentration on the establishment of steady-state in continuous ethanol fermentation of molasses. *Biomass*. 1985;6:247–256.
11. Mulchandani AB, Volesky. Modelling of the acetone-butanol fermentation with cell retention. *Can J Chem Eng*. 1986;64:625–631.
12. Ferras E, Minier M, Goma G. Acetonobutylic fermentation: improvement of performances by coupling continuous fermentation and ultrafiltration. *Biotechnol Bioeng*. 1986;28:523–533.
13. Beavan M, Zawadzki B, Droniuk R, Lawford H, Fein J. Comparative performance trials with yeast and *Zymomonas* for fuel alcohol production from corn. *Appl Biochem Biotechnol*. 1989;20/21:319–326.
14. Jarzebski AB. Modeling oscillatory behavior in continuous ethanol fermentation. *Biotechnol Lett*. 1992;14:137–142.
15. Daugulis AJ, McLellan PJ, Li J. Experimental investigation and modeling of oscillatory behavior in continuous cultures of *Zymomonas mobilis*. *Biotechnol Bioeng*. 1997;56:99–105.
16. Mcllean PJ, Daugulis AJ, Li J. The incidence of oscillatory behavior in the continuous behavior of *Zymomonas mobilis*. *Biotechnol Prog*. 1999;15;667–680.
17. Garhyan P, Elnshaie S. Static/dynamic bifurcation and chaotic behavior of an ethanol fermentor. *Ind Eng Chem Res*. 2004;43:1260–1273.
18. Mahecha Botero A, Garhyan P, Elnashie SSEH. Nonlinear characterestics of a membrane fermentor for ethanol production and their implications. *Nonlinear Anal Real World Appl*. 2006;7:432–457.
19. Parulekar SJ, Semones GB, Rolf MJ, Lievense JC, Lim HC. Introduction and elimination of oscillations in continuous cultures of *Saccharomyces cerevisiae*. *Biotechnol Bioeng*. 1986;28:700–710.
20. Strassle C, Sonnleitner B, Fiechter AA. A predictive model for the for the spontaneous synchronization of *Saccharomyces cerevisiae* grown in continuous culture, II. Experimental verification. *J Biotechnol*. 1989;9:191–208.
21. Von Meyenberg HK. *Stable synchrony oscillations in continuous culture of* Saccharomyces cerevisiae *under glucose limitation*. In: Chance B, Pye EK, Shosh AK, Hess B, editors. *Biological and Biochemical Oscillators*. New York: Academic Press, 1973:411–417.
22. Jones KD, Kompala DS. Cybernetic model of the growth dynamics of the *Saccharomyces cerevisiae* in batch and continuous cultures. *J Biotechnol*. 1999;71:105–131.
23. Zhang Y, Henson MA. Bifurcation analysis of continuous biochemical reactor models. *Biotechnol Prog*. 2001;17:647–660.
24. Simpson DJW, Kompala DS, Meiss JD. Discontinuity induced bifurcations in a model of *Saccharomyces cerevisiae*. *Math Biosci*. 2009;218:40–49.
25. Dhooge A, Govearts W, Kuznetsov AY. MATCONT: a Matlab package for numerical bifurcation analysis of ODEs. *ACM Trans Math Softw*. 2003;29:141–164.
26. Dhooge A, Govaerts W; Kuznetsov AY, Mestrom W, Miet AM. *CL_MATCONT*; A continuation toolbox in Matlab, 2004.

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