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# A note on competition in the bioreactor with toxin

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In this paper, we investigate a model with yields: $\gamma_1 = A_1 + B_1 S^m$ and $\gamma_2 = A_2 + B_2 S^n$, for the competition in the bioreactor of two competitors for a single nutrient, in which one of the competitors produces toxin against its opponent. The existence of limit cycles in the 3-D system is obtained by using a Hopf bifurcation.

**KEY WORDS:** Bioreactor, variable yields, toxin, bifurcation, limit cycles

**AMS subject classification:** 34C35, 34D20, 92D25

## 1. Introduction

Modeling microbial growth is an interesting topic in mathematical chemistry and mathematical biology [1]. Most of the models in bioreactors assume that no toxin produced by one organism to inhibit the other. However, in nature microorganisms often produce inhibitors against their rivals. Thus, considering the effect of anti-competitor toxin in modeling bioreactors is necessary [2–7]. In the earlier years, models of bioreactor assumed that yield coefficients are constants [1]. Later, it was discovered that constant yields failed to describe the nonlinear phenomena such as oscillation, time-delay and chaos in the reactions. Many authors tried to modify the model, in which one is using variable yields instead of constant yields [8–14]. Recently, a model with general quadric yields of competition in the bioreactor of two competitors for a single nutrient, where one of the competitors produces toxin against its opponent is studied [3]. In this note, we use a quite similar idea to report the results for the competition model with variable yields: $\gamma_1 = A_1 + B_1 S^m$ and $\gamma_2 = A_2 + B_2 S^n$, where $m, n$ are non-negative integers. In addition, by using a 3-D Hopf bifurcation, we prove the existence of limit cycles. Models with variable yields are studied in many references (see [8–10,13,14], for instance).

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The existence of periodic solutions of the $n-$dimensional differential system for $n \geqslant 3$ is interesting in both theory and applications. This is because that the situation of $n \geqslant 3$ is much complicated than the one of $n = 2$ due to the powerful tools in the plane system like Poincare–Bendixson theorem cannot be applied directly to the cases of $n \geqslant 3$. Some counterexamples can be found in D'Heedene [15] and Schweitzer [16]. Thus, any results regarding the limit cycles in $3^+$-D systems are welcomed in the study of nonlinear dynamic systems.

## 2. Main results

The model considered in this paper is

$$\begin{aligned} S' &= 1 - S - \frac{x}{A_1 + B_1 S^m}\frac{m_1 S}{a_1 + S} - \frac{y}{A_2 + B_2 S^n}\frac{m_2 S}{a_2 + S}, \\ x' &= x\left(\frac{m_1 S}{a_1 + S} - 1 - y\frac{k\gamma}{1-k}\right), \\ y' &= y\left((1-k)\frac{m_2 S}{a_2 + S} - 1\right), \end{aligned} \tag{1}$$

where, $S(t)$ denote the concentration of nutrient in the bioreactor, $x(t)$, the concentration of the toxin sensitive microorganism, $y(t)$, the toxin-producing organism, $m_i$, the maximal growth rate, $a_i$, the Michaelis–Menten constant, $i = 1, 2$; $A_1 + B_1 S^m$ and $A_2 + B_2 S^n$, are the yields, where $m, n$ are non-negative integers, and $A_i > 0, B_i \geqslant 0, i = 1, 2$. The intersection between the toxin and the sensitive microorganism is taken to be of mass action form: $-\gamma x$, where $\gamma$ is a non-negative parameter. The constant $k$ represents the fraction of potential growth devoted to produce the toxin; $k = 0$ means a system asymptotic to the standard bioreactor and $k = 1$ represents all effects devoted to producing the toxin and results in no growth and thus extinction. Usually, it is assumed that $0 < k < 1$. One can find the idea how to derive the model in [7].

Denote

$$\lambda_1 = \frac{a_1}{m_1 - 1}, \qquad \lambda_2 = \frac{a_2}{(1-k)m_2 - 1}, \qquad \hat{\lambda} = \varphi^{-1}(0), \tag{2}$$

where

$$\varphi(\lambda) = \frac{m_1\lambda}{a_1 + \lambda} - 1 - k\gamma(1-\lambda)(A_2 + B_2 S^n), \tag{3}$$

where $\varphi(\lambda)$ is an increasing function and there exists a $\hat{\lambda}$ such that $\varphi(\hat{\lambda}) = 0$, $\hat{\lambda} \in (\lambda_1, 1)$.

System (1) has four possible equilibrium points:

$$E_0(1,0,0), \qquad E_1\big(\lambda_1, (1-\lambda_1)(A_1+B_1\lambda_1^m), 0\big), \quad \text{if } \lambda_1 < 1,$$
$$E_2\big(\lambda_2, 0, (1-k)(1-\lambda_2)(A_2+B_2\lambda_2^n)\big), \quad \text{if } \lambda_2 < 1, \text{ and}$$
$$E_3\big(\lambda_2, x^*, y^*\big), \quad \text{if } \lambda_1 < \lambda_2 < \hat{\lambda},$$

where,

$$x^* = \frac{(A_1+B_1\lambda_2^m)(a_1+\lambda_2)}{m_1\lambda_2}\left(1-\lambda_2-\frac{1}{k\gamma(A_2+B_2\lambda_2^n)}\left(\frac{m_1\lambda_2}{a_1+\lambda_2}-1\right)\right),$$
$$y* = \frac{1-k}{k\gamma}\left(\frac{m_1\lambda_2}{a_1+\lambda_2}-1\right) \text{ (both are positive).} \tag{4}$$

Denote

$$R_1 \equiv \frac{(1-\lambda_1)\left(m\lambda_1^{m-1}(a_1+\lambda_1)^2-\lambda_1^m m_1 a_1\right)-\lambda_1^m(a_1+\lambda_1)^2}{(a_1+\lambda_1)^2+m_1a_1(1-\lambda_1)},$$
$$R_2 \equiv \frac{(1-k)(1-\lambda_2)\left(n\lambda_2^{n-1}(a_2+\lambda_2)^2-\lambda_2^n m_2 a_2\right)-\lambda_2^n(a_2+\lambda_2)^2}{(a_2+\lambda_2)^2+m_2a_2(1-k)(1-\lambda_2)}. \tag{5}$$

It is easy to prove the following theorems (see, e.g., [3,7]).

**Theorem 1.** (i) $E_0$ always exists. It is locally asymptotically stable if $\lambda_i > 1, i = 1,2$, and unstable if either inequality is reversed. (ii) $E_1$ exists if $\lambda_1 < 1$ with a 2-D stable manifold: $y = 0$; and it is locally asymptotically stable if $\lambda_1 < \lambda_2$ and $A_1/B_1 > R_1$, and unstable if either inequality is reversed. (iii) $E_2$ exists if $\lambda_2 < 1$. If it exists, it has a 2-D stable manifold: $x = 0$, and it is locally asymptotically stable if $\lambda_2 < \hat{\lambda}$, and $A_2/B_2 > R_2$, and unstable if either inequality is reversed. (iv) $E_3$ exists if $\lambda_1 < \lambda_2 < \hat{\lambda}$, and if it exists it is always unstable with a 2-D stable manifold.

**Theorem 2.** (i) If $\lambda_1 < \lambda_2$ and $A_1/B_1 > R_1$, then $E_1$ is globally asymptotically stable. (ii) If $\lambda_2 < \hat{\lambda}$ and $A_2/B_2 > R_2$, then $E_2$ is globally asymptotically stable.

Like in the case of the variable yields $\gamma_i = A_i + B_iS + C_iS^2, i = 1,2$ ([3]), by the standpoint of the operation, the reactor is not functioning as desired if $E_0$ or $E_1$ is globally asymptotically stable, in which $\lim_{t\to\infty} y(t) = 0$. Conversely, if $E_2$ is asymptotically stable, $y$ survives and it is manufacturing the desired product. Therefore, conditions that guarantee the existence of an oscillation or a limit cycle around the equilibrium point $E_2$ are essential to the production of the bioreactor.

Following the argument of theorem 5 ([7]), we have

**Theorem 3.** There is a positive invariant set of system (1) in the positive octant, which takes the form

$$\Omega^+ = \{(S, x, y) | 0 \leqslant S \leqslant L - x - y, 0 \leqslant x \leqslant (1 - \lambda_1)(A_1 + B_1\lambda_1^m) + \varepsilon_0,$$
$$0 \leqslant y \leqslant (1 - k)(1 - \lambda_2)(A_2 + B_2\lambda_2^n) + \varepsilon_0, \varepsilon_0 = \text{ const. } L >> 1\}.$$

The following theorem is for the global stability of $E_2$, and in the proof a generalized Liapunov function and the LaSalle corollary are used (see [3,7,17]).

**Theorem 4.** If $\lambda_2 < \lambda_1$, and assume

$$B_2m_2(\lambda_2 + \lambda_2^2 + \cdots + \lambda_2^n)\left((1 - k)(1 - \lambda_2)(A_2 + B_2\lambda_2^n) + \varepsilon_0\right)$$
$$- A_2a_2(A_2 + B_2\lambda_2^n) \leqslant 0, \tag{6}$$

then the equilibrium $E_2$ is globally asymptotically stable.

*Proof.* Let

$$V(S, x, y) = \int_{\lambda_2}^{S} \frac{\eta - \lambda_2}{\eta} \mathrm{d}\eta + c_1 \int_{\hat{y}}^{y} \frac{\eta - \hat{y}}{\eta} \mathrm{d}\eta + c_2x,$$

where $c_1, c_2$ are determined later, and $\hat{y} = (1 - k)(1 - \lambda_2)(A_2 + B_2\lambda_2^n)$.
Then

$$V' = \frac{S - \lambda_2}{S}\left(1 - S - \frac{x}{A_1 + B_1S^m}\frac{m_1S}{a_1 + S} - \frac{y}{A_2 + B_2\lambda_2^n}\frac{m_2S}{a_2 + S}\right)$$
$$+c_1\frac{y - \hat{y}}{y}y\left((1 - k)\frac{m_2S}{a_2 + S} - 1\right) + c_2x\left(\frac{m_1S}{a_1 + S} - 1 - y\frac{k\gamma}{1 - k}\right).$$

It follows that

$$V' = \left(\frac{S - \lambda_2}{S}(1 - S) - c_1\hat{y}\left((1 - k)\frac{m_2S}{a_2 + S} - 1\right)\right)$$
$$+\left(-\frac{S - \lambda_2}{S}\frac{y}{A_2 + B_2\lambda_2^n}\frac{m_2S}{a_2 + S} + c_1y\left((1 - k)\frac{m_2S}{a_2 + S} - 1\right)\right)$$
$$- c_2\frac{xyk\gamma}{1 - k} + c_2x\left(\frac{m_1\lambda_2}{a_1 + \lambda_2} - 1\right)$$
$$+x\left(c_2\left(\frac{m_1S}{a_1 + S} - \frac{m_1\lambda_2}{a_1 + \lambda_2}\right) - \frac{S - \lambda_2}{S}\frac{1}{A_1 + B_1S^m}\frac{m_1S}{a_1 + S}\right)$$
$$\equiv V_1 + V_2 + V_3 + V_4 + V_5.$$

Note that

$$(1-k)\frac{m_2 S}{a_2+S}-1=\frac{((1-k)m_2-1)\,(S-\lambda_2)}{a_2+S},\ \text{and}$$

$$1-k=\frac{a_2+\lambda_2}{m_2\lambda_2}.$$

We can determine the sign of each part of $V'$as follows:

First choose $c_1=\frac{m_2}{(A_2+B_2\lambda_2^n)((1-k)m_2-1)}$. Then it follows that

$$\begin{aligned}V_1&=\frac{S-\lambda_2}{S}(1-S)-c_1\hat{y}\frac{((1-k)m_2-1)(S-\lambda_2)}{a_2+S}\\&=(S-\lambda_2)\left(\frac{1-S}{S}-m_2(1-k)\frac{1-\lambda_2}{a_2+S}\right)\\&=(S-\lambda_2)\left(\frac{1-S}{S}-m_2\frac{a_2+\lambda_2}{m_2\lambda_2}\frac{1-\lambda_2}{a_2+S}\right)\\&=-(S-\lambda_2)^2\frac{a_2+S\lambda_2}{\lambda_2 S(a_2+S)}\leqslant 0.\end{aligned}$$

Therefore

$$\begin{aligned}V_2&=-\frac{S-\lambda_2}{S}\frac{y}{A_2+B_2S^n}\frac{m_2S}{a_2+S}+c_1y\left((1-k)\frac{m_2S}{a_2+S}-1\right)\\&=\frac{S-\lambda_2}{S}\frac{y}{A_2+B_2S^n}\frac{m_2S}{a_2+S}+\frac{m_2}{(A_2+B_2\lambda_2^n)((1-k)m_2-1)}\\&\quad y\frac{(1-k)m_2-1}{a_2+S}(S-\lambda_2)\\&=\frac{m_2(S-\lambda_2)}{a_2+S}y\left(\frac{1}{A_2+B_2\lambda_2^n}-\frac{1}{A_2+B_2S^n}\right)\\&=\frac{ym_2(S-\lambda_2)^2}{a_2+S}\frac{B_2(S^{n-1}+S^{n-2}\lambda_2+\cdots+\lambda_2^{n-1})}{(A_2+B_2\lambda_2^n)(A_2+B_2S^n)}.\end{aligned}$$

If

$$\frac{(S-\lambda_2)^2}{a_2+S}\left(\frac{ym_2B_2(S^{n-1}+S^{n-2}\lambda_2+\cdots+\lambda_2^{n-1})}{(A_2+B_2S^n)(A_2+B_2\lambda_2^n)}-\frac{a_2+S\lambda_2}{\lambda_2 S}\right)\leqslant 0,$$

then $V_1 + V_2 \leqslant 0$. That is,

$$\frac{yS(S^{n-1} + S^{n-2}\lambda_2 + \cdots + \lambda_2^{n-1})}{(A_2 + B_2 S^n)(a_2 + S\lambda_2)} - \frac{A_2 + B_2\lambda_2^n}{B_2 m_2 \lambda_2} \leqslant 0,$$

$$\frac{S^n + S^{n-1}\lambda_2 + \cdots + S\lambda_2^{n-1})}{(A_2 + B_2 S^n)(a_2 + S\lambda_2)} - \frac{A_2 + B_2\lambda_2^n}{B_2 m_2 \lambda_2 \left((1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n) + \varepsilon_0\right)} \leqslant 0.$$

Therefore, the assumption (6) implies that $V_1 + V_2 \leqslant 0$.

Moreover, if choose $c_2 = \frac{a_1+\lambda_2}{a_1(A_1+B_1S^m)}$,

$$V_3 = -c_2 \frac{xyk\gamma}{1-k} \leqslant 0 \left(\text{since } c_2 = \frac{a_1 + \lambda_2}{a_1(A_1 + B_1 S^m)} \geqslant 0\right),$$

$$V_4 = c_2 x \left(\frac{m_1\lambda_2}{a_1 + \lambda_2} - 1\right) < 0 \left(\text{since } c_2 = \frac{a_1 + \lambda_2}{a_1(A_1 + B_1 S^m)} \geqslant 0, \lambda_2 < \lambda_1\right), \text{ and}$$

$$\begin{aligned} V_5 &= x\left(c_2\left(\frac{m_1 S}{a_1 + S} - \frac{m_1\lambda_2}{a_1 + \lambda_2}\right) - \frac{S - \lambda_2}{S}\frac{1}{A_1 + B_1 S^m}\frac{m_1 S}{a_1 + S}\right) \\ &= \frac{x m_1(S - \lambda_2)}{a_1 + S}\left(c_2\frac{a_1}{a_1 + \lambda_2} - \frac{1}{A_1 + B_1 S^m}\right) \\ &= 0. \end{aligned}$$

Note that since $c_2 = \frac{a_1+\lambda_2}{a_1(A_1+B_1S^m)}$, which is a function of $S$, there is one more term in $V'$ created by differentiating, that is

$$\begin{aligned} V_6 &= \left(\frac{\mathrm{d}c_2}{\mathrm{d}S}\right)x = \left(\frac{a_1 + \lambda_2}{a_1(A_1 + B_1 S^m)}\right)' x \\ &= -\frac{(a_1 + \lambda_2)B_1 m S^{m-1}}{a_1(A_1 + B_1 S^m)^2}x \\ &\leqslant 0. \end{aligned}$$

Therefore, $V' < 0$.

By the LaSally corollary, all trajectories tend to the largest invariant set in $\Delta = \{(S, x, y) | V' = 0\}$. This requires $S \equiv \lambda_2$ and $x \equiv 0$.

To make $\{S | S = \lambda_2\}$ invariant under the condition $x = 0$, it requires

$$S' = 1 - \lambda_2 - y\frac{1}{(1-k)(A_2 + B_2\lambda_2^n)} = 0.$$

This implies $y = (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n)$. Therefore $\{E_2\}$ is the only invariant set in $\Delta$. We thus complete the proof of theorem 4.

Let $\mu = R_2 - A_2/B_2$ be the bifurcation parameter. It is easy to see that system (1) can be written in the parameter $\mu$ as follows:

$$S' = F_1(S, x, y, \mu),$$
$$x' = F_2(S, x, y, \mu),$$
$$y' = F_3(S, x, y, \mu).$$

Use the variable change:

$$\bar{S} = S - \lambda_2, \qquad \bar{x} = x, \qquad \bar{y} = y - (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n)$$

system (1) can be written in variables $\bar{S}, \bar{x}, \bar{y}$, as

$$\frac{\mathrm{d}X}{\mathrm{d}t} = f(X, \mu), \tag{7$-\mu$}$$

whose Jacobian is denoted as $J(\bar{S}, \bar{x}, \bar{y})$.

We then introduce the following lemma ([3, 7, 17]) and the definition of derived operator, which are need in the proof of theorem 5.

**Lemma 1.** Let $W$ be an open set in $R^3$, $(0, 0, 0) \in W$, and $f: W \times (-\mu_0, \mu_0) \to R^3$ be an analytic function on $W \times (-\mu_0, \mu_0)$, where $\mu_0$ is a small positive number. Denote the Jacobian of $f$ at $(X, \mu) = ((0, 0, 0), 0)$ as $J(f(0, 0))$. Assume

(i) system $(7) - \mu$ has $(0, 0, 0)$ as its equilibrium point for any $\mu$;

(ii) the eigenvalues of $J(f(0, 0))$: $\pm i\beta(\mu)|_{\mu=0} = \pm i\beta(0), \delta(\mu)|_{\mu=0} = \delta(0)$ satisfy the conditions $\beta(0) > 0, \delta(0) < 0$.

Then, if $(0, 0, 0)$ is asymptotically stable at $\mu = 0$, unstable on $\mu > 0$, there exists a sufficiently small $\mu, \mu > 0$ such that system $(7){-}\mu$ has an asymptotically stable closed orbit surrounding $(0, 0, 0)$.

The proof of lemma 1 is listed in the appendix.

**Definition 1.** Let $f$ be a vector function from $R^n$ to $R^m$. $f$ is said to be differentiable at point $a \in R^n$, if there exists a linear operator $\wp : R^n \to R^m$, such that

$$f(a + h) - f(a) = \wp h + \mathrm{o}(h) \quad (h \in R^n).$$

Then $\wp$ is called the derived operator of $f(x)$ at $a$, denoted as $\wp = Df(a)$.

It is easy to see that if $f(x)$ is differentiable at $a$, then $\frac{\partial f_i}{\partial x_j}\Big|_{x=a}$ exists for $i = 1, 2, \ldots, m,\ j = 1, 2, \ldots, n$. Note that the derived operator $Df(a)$ is just the variational matrix:

$$\begin{pmatrix} \frac{\partial f_1}{\partial x_1} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \cdots & \cdots & \cdots \\ \frac{\partial f_m}{\partial x_1} & \cdots & \frac{\partial f_m}{\partial x_n} \end{pmatrix}_{x=a}$$

of system $\frac{dX}{dt} = f(X, \mu)$.

We now prove the three dimensional Hopf bifurcation theorem for system (1).

**Theorem 5.** If $\lambda_2 < \lambda_1$, and (6) holds, then system (1) undergoes a Hopf bifurcation at $\mu = 0$, (that is, $R_2 = A_2/B_2$), and the periodic solution created by the Hopf bifurcation is asymptotically stable for $0 < R_2 - A_2/B_2 << 1$.

*Proof.* Consider system (7)$-\mu$ and its Jacobian at $(\bar{S}, \bar{x}, \bar{y}) = (0, 0, 0)$

$$J(f(0,0)) = J(\bar{S}, \bar{x}, \bar{y})\Big|_{\substack{(\bar{S}, \bar{x}, \bar{y}) = (0,0,0) \\ \mu = 0}} = J(S, x, y)\Big|_{\substack{(S,x,y) = (\lambda_2, 0, (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n)) \\ R_2 = A_2/B_2}}.$$

Its characteristic equation has the eigenvalues: $\pm i\beta(0)$ and $\delta(0)$, where

$$\beta(0) = \frac{1}{a_2 + \lambda_2}\sqrt{(1-k)(1-\lambda_2)m_2 a_2} > 0,$$
$$\delta(0) = \varphi(\lambda_2) < 0 \quad (\text{since } \lambda_2 < \lambda_1 < \hat{\lambda}).$$

The hypotheses of lemma 1 are satisfied. From theorem 4, it follows that:

(1) The equilibrium of system (1): $(0, 0, 0)$ in the $\bar{S}, \bar{x}, \bar{y}$ coordinate system, or $(\lambda_2, 0, (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n))$ in $S, x, y$, is globally asymptotically stable at $\mu < 0$ and $\mu$=0 (even in the case of $A_2/B_2 = R_2$, see the proof of theorem 4);

(2) $(0, 0, 0)$ in $\bar{S}, \bar{x}, \bar{y}$ coordinates, or $(\lambda_2, 0, (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n))$ in $S, x, y$, is unstable if $\mu > 0$ (since $A_2/B_2 < R_2$ theorem 2-(iii) implies the instability).

Therefore, system (7)$-\mu$ undergoes a Hopf bifurcation at $\mu = 0$, and so does system (1) at $A_2/B_2 = R_2$. From lemma 1 it proves, for a sufficient small $\mu, \mu > 0$, system (7)$-\mu$ has an asymptotically stable closed orbit surrounding $(0, 0, 0)$. In other words, for $0 < R_2 - A_2/B_2 << 1$, system (1) has an asymptotically stable closed orbit surrounding $E_2(\lambda_2, 0, (1-k)(1-\lambda_2)(A_2 + B_2\lambda_2^n))$. Theorem 5 is obtained.

## 3. Conclusion

The study of the competition in the bioreactor with one organism producing toxins has been interesting to many authors [2,9–12]. However, most of these models are assumed that the yields are constants. The recent paper of the authors [3] considered a model with general quadric yields of competition in the bioreactor of two competitors for a single nutrient where one of the competitors can produce toxin against its opponent. Here, we use the same idea for a similar model but different and more general variable yields. Under different conditions, we derived the existence of limit cycles. This implies the competition system with toxin involved may have at least two limit cycles. Results of this paper and [3] are not include with each other. Also, in [3], there are some misprints such as in formula (5) $\lambda_1 = \frac{a}{m_1 - 1}$ should be replaced by $\frac{a_1}{m_1 - 1}$; in formula (14) $\lambda_1$ should be $\lambda_2$, and theorem 4 of [3] needs some modification just like, we have done in Theorem 4 in section 2.

We would also like to add lemma 1 and its proof in Appendix since the corollary of center manifold theorem is very useful in studying 3-D bifurcations. The main idea is based on a Chinese reference [18].

## Appendix

*Proof of lemma 1.* Let $O(0, 0, 0)$ be the origin. By the hypothesis of lemma 1, for sufficiently small $\mu > 0$, the derived operator of $f(X, \mu)$ at $X = (0, 0, 0)$ and $\mu = 0$ takes the form: $Df(O, 0)$ with the eigenvalues: $\alpha(\mu) \pm i\beta(\mu)$ and $\delta(\mu)$. It is not difficult to see that $\alpha(0) = 0, \beta(0) > 0, \delta(\mu) < \bar{\delta} < 0$, where $\bar{\delta}$ is some constant. For any $\mu$, $Df(O, \mu) = Jf(O, \mu)$, which is the variational matrix of system $(7) - \mu$.

We first assume that $Df(O, \mu)$ has the standard form:

$$Df(O, \mu) = \begin{pmatrix} \alpha(\mu) & -\beta(\mu) & 0 \\ \beta(\mu) & \alpha(\mu) & 0 \\ 0 & 0 & \delta(\mu) \end{pmatrix}. \tag{8}$$

Consider the 4-D differential equations

$$\begin{aligned} \dot{x}_1 &= f_1(x_1, x_2, x_3, \mu), \\ \dot{x}_2 &= f_2(x_1, x_2, x_3, \mu), \\ \dot{x}_3 &= f_3(x_1, x_2, x_3, \mu), \\ \dot{\mu} &= f_4(x_1, x_2, x_3, \mu) \equiv 0. \end{aligned} \tag{9}$$

For the simplicity, we denote the right-hand side functions of (9) as $f(X, \mu)$, whose derived operator at $X = (0, 0, 0)$, $\mu = 0$ takes the form

$$Df((0,0,0),0) = \begin{pmatrix} 0 & -\beta(0) & 0 & 0 \\ \beta(0) & 0 & 0 & 0 \\ 0 & 0 & \delta(0) & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix},$$

which has the eigenvalues: $+i\beta(0)$, $-i\beta(0)$, $\delta(0)$, and 0.

Let $\varphi_t(X, \mu)$ be a flow of equation (9). It follows that

$$\frac{\mathrm{d}}{\mathrm{d}t} D\varphi_t(X,\mu) = Df(X,\mu)\big|_{(X,\mu)=\varphi_t(X,\mu)} D\varphi_t(X,\mu).$$

Since $(X, 0) = (O, 0)$ is an equilibrium of system (9), the matrix satisfies the following differential equation

$$\frac{\mathrm{d}}{\mathrm{d}t} D\varphi_t(O,0) = Df(O,0) \cdot D\varphi_t(O,0). \tag{10}$$

Note that $D\varphi_0(O, 0) = I$, the identity matrix. Therefore, $D\varphi_t(O, 0)$ is the fundamental solution matrix of the linear equation $\dot{x} = Df(O, 0)x$. That is

$$D\varphi_t(O,0) = e^{Df(O,0)t} = \begin{pmatrix} \cos\beta(0)t & -\sin\beta(0)t & 0 & 0 \\ \sin\beta(0)t & \cos\beta(0)t & 0 & 0 \\ 0 & 0 & e^{\delta(0)t} & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix},$$

which implies that $D\varphi_t(O, 0)$ has eigenvalues $e^{\pm i\beta(0)t}$, $e^{\delta(0)t}$, and 1. In summary, for the flow $\varphi_t(X, \mu)$ all the conditions of the center manifold theorem are satisfied. Let $H$ be the three-dimensional subspace spanned by the eigenvalues $e^{\pm i\beta(0)t}$ and 1 of $D\varphi_t(O, 0)$ in the unit circle, then it follows that $H$ is $x_3 = 0$. By the center manifold theorem, there exists a neighborhood $\bar{V}$ of the origin $\bar{O}((0, 0, 0), 0)$, and a three dimensional face $M$. $M$ is smooth to any degree since the flow $\varphi_t(X, \mu)$ is analytic, with $\bar{O} \in M \subset \bar{V}$, and $M$ is tangent to $H$ at the origin. Therefore

(1) if $\varphi_t(X, \mu) \in \bar{V}$ for $(X, \mu) \in M$, $t > 0$, then $\varphi_t(X, \mu) \in M$;

(2) if $\varphi_{nt}(X, \mu) \in \bar{V}$ for $t > 0$ and $n = 0, 1, 2, \ldots$, then the distance between $\varphi_{nt}(X, \mu)$ and $M$ tends to zero when $n \to \infty$.

Since $M$ and $H$ are tangent to each other, we can denote the equation of $M$ as $x_3 = x_3(x_1, x_2, \mu)$. Let $\mu_0$ be sufficiently small, and $M_{\mu_0}$ denote the intersection of $M$ and the plane $\mu = \mu_0$. It easy to see that $M_{\mu_0}$ is the 2-D surface $x_3 = x_3(x_1, x_2, \mu_0)$, and thus we obtain a set of 2-D surfaces $M_\mu \subset R^3$.

It follows that $M_\mu$ has two properties:

(i) for sufficiently small $\mu_0$, the point $(x_1, x_2, x_3) = (0, 0, 0)$ is on $M_{\mu_0}$, that is $x_3(0, 0, \mu_0)$=0. This is because if $\mu_0$ is sufficiently small, $(0, 0, 0, \mu_0) \in \bar{V}$. Moreover, $(0, 0, 0, \mu_0)$ is the equilibrium of equation (9). Therefore, for $t > 0, n = 0, 1, 2, \ldots, \varphi_{nt}(0, 0, 0, \mu_0) = (0, 0, 0, \mu_0) \in \bar{V}$. The center manifold theorem implies

$$\lim_{n\to\infty} \varphi_{nt}(0, 0, 0, \mu_0) = (0, 0, 0, \mu_0) \in M,$$

so $x_3(0, 0, \mu_0) = 0$ and $(0, 0, 0) \in M_{\mu_0}$;

(ii) if $(X, \mu_0) \in M$, then $\varphi_t(X, \mu_0) \in \bar{V}$ for $t > 0$. The center manifold theorem indicates $\varphi_t(X, \mu_0) \in M$. Since $\frac{d\mu}{dt} = 0$, $\mu$ remains unchanged while $t$ varies. Thus for $t > 0\, \varphi_t(X, \mu_0) \in M_{\mu_0}$. In other words, for sufficiently small $\mu$, any trajectory of system (7)$-\mu$ stays in $M_\mu$ if it starts from a point near the origin $(0, 0, 0) \in M_{\mu_0}$.

Since for any point in $M_\mu$, there is a trajectory of (7)$-\mu$ in $M_\mu$, the normal of $M_\mu$ is perpendicular to the vector field of $f(X, \mu)$. It follows that the right-hand side function of the equation $x_3 = x_3(x_1, x_2, \mu)$ in $M_\mu$ satisfies the partial differential equation

$$f_1(x_1, x_2, x_3, \mu)\frac{\partial x_3}{\partial x_1} + f_2(x_1, x_2, x_3, \mu)\frac{\partial x_3}{\partial x_2} - f_3(x_1, x_2, x_3, \mu) = 0.$$

Substituting $x_3 = x_3(x_1, x_2, \mu)$ into the first two equations of (7)$-\mu$, we obtain the following 2-D system in $R^2$:

$$\begin{aligned} \dot{x}_1 &= f_1(x_1, x_2, x_3(x_1, x_2, \mu), \mu), \\ \dot{x}_2 &= f_2(x_1, x_2, x_3(x_1, x_2, \mu), \mu). \end{aligned} \tag{11$-\mu$}$$

Note, that the trajectory of $(11) - \mu$ is the projection of the trajectory of (7)-$\mu$ in $M_\mu$ onto the $x_1 - x_2$ plane, and for sufficiently small $\mu$, $x_3(0, 0, \mu) = 0$. Thus, for sufficiently small $\mu$, $(0, 0)$ is an equilibrium of system $(11) - \mu$. The variational matrix of $(11) - \mu$ at $(0, 0)$ is

$$\begin{pmatrix} \alpha(\mu) & -\beta(\mu) \\ \beta(\mu) & \alpha(\mu) \end{pmatrix}.$$

Since $\alpha(0)$ is zero, thus $(0, 0)$ is a center of system $(11) - 0$.

By the hypothesis of lemma 1, $(0, 0, 0)$ is an asymptotically stable equilibrium, we can use a similar argument of the formal series method to the equilibrium $(0, 0, 0)$ of system $(7) - 0$ and derive an integer $2m$ such that $C_3 = \cdots = C_{2m-1} = 0$, but $C_{2m} < 0$. If the central manifold $M$ is smooth enough, for the equilibrium $(0, 0)$ of system $(11) - 0$, there must exist an integer $2m$ such that $C_3 = \cdots = C_{2m-1} = 0$, but $C_{2m} < 0$, and a function $\Phi(x_1, x_2)$ with

$$\left.\frac{\mathrm{d}\Phi}{\mathrm{d}t}\right|_{(11)-0} = C_{2m}(x_1^2 + x_2^2)^m$$
$$+(\text{terms of } x_1, x_2 \text{ with powers of } 2m + 1 \text{ or higher}).$$

Therefore, $(0, 0)$ is a stable focus of system $(11) - 0$.

Since the hypothesis of the lemma assumes that $(0, 0, 0)$ is an unstable equilibrium for $\mu > 0, \mu$ is sufficiently small, $\alpha(\mu)$ cannot be negative. If $\alpha(\mu) > 0$, $(0, 0)$ is an unstable equilibrium of system $(11) - \mu$; if $\alpha(\mu) = 0$, following the same argument of the formal series method, there exists an integer $k(\mu)$ such that $C_3 = \cdots = C_{k(\mu)-1} = 0$, but $C_{k(\mu)} > 0$. Let $k_0$, a positive integer, be an upper bound of $k(\mu)$. The existence of $k_0$ is ensured by the fact that, for $\mu_0$ and $\mu$, sufficiently close to $\mu_0$, $k(\mu) \leqslant k(\mu_0)$. Suppose $M$ is smooth enough (continuously differentiable up to $k_0+1$'s time). When we use the formal series method to the equilibrium $(0, 0)$ of $(11)-\mu$, we have $C_3 = \cdots = C_{k(\mu)-1} = 0$, but $C_{k(\mu)} > 0$. Thus, the origin $(0, 0)$ is an unstable equilibrium of system $(11) - \mu$.

In order to prove that there exists a stable limit cycle around $(0, 0)$ of system $(11) - \mu$ for sufficiently small $\mu > 0$, we use the linear transform

$$x_1 = au + bv,$$
$$x_2 = cu + dv$$

and transfer system $(11) - 0$ to

$$\begin{aligned}\dot{u} &= -v + U_2(u, v) \equiv U(u, v, 0),\\ \dot{v} &= u + V_2(u, v) \equiv V(u, v, 0)\end{aligned} \tag{12–0}$$

and system $(11) - \mu$ to

$$\begin{aligned}\dot{u} &= U(u, v, \mu),\\ \dot{v} &= V(u, v, \mu).\end{aligned} \tag{12$-\mu$}$$

Since $(0, 0)$ is a stable central focus of system $(11) - 0$, and it is also a stable central focus of system (12)–0. By the formal series method for the center, there exists a function

$$F(u, v) = u^2 + v^2 + F_3(u, v) + \cdots + F_{2k_0}(u, v),$$

such that along with the trajectory of system (12)–0,

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0} = -C_0(u^2 + v^2)^{k_0} + (\text{terms of } u, v \text{ with power } 2k_0 + 1 \text{ or higher}),$$

where $C_0$ is a positive constant.

Rewrite the above equation as

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0} = -\frac{C_0}{2}(u^2+v^2)^{k_0} + (u^2+v^2)^{k_0}\left(-\frac{C_0}{2} + \mathrm{o}\left(\sqrt{u^2+v^2}\right)\right). \quad (13)$$

It follows that there exists an $r_0 > 0$, such that for $u^2+v^2 \leqslant r_0^2$, $-\frac{C_0}{2} + \mathrm{o}\left(\sqrt{u^2+v^2}\right) < 0$. Let $F(u,v) = a_0$ be an isocline of the function $F(u,v)$ in the region of $u^2+v^2 \leqslant r_0^2$, and let $u^2+v^2 = r_1^2$ be a circle inside the isocline. We estimate $\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-\mu}$ in the annular region $r_1^2 \leqslant u^2+v^2 \leqslant r_0^2$ as follows.

Since

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-\mu} = \left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0} + \left(\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-\mu} - \left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0}\right) \quad (14)$$

by (13), in the annular region $r_1^2 \leqslant u^2+v^2 \leqslant r_0^2$, the first term of the right-hand side of (14) satisfies the following inequality

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0} < -\frac{C_0}{2} r_1^{2k}. \quad (15)$$

For the second-term of the right-hand side of (14),

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-\mu} - \left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-0} = \frac{\partial F}{\partial u}\left(U(u,v,\mu) - U(u,v,0)\right) + \frac{\partial F}{\partial v}\left(V(u,v,\mu)\right.$$
$$\left. - V(u,v,0)\right).$$

Since $\frac{\partial F}{\partial u}, \frac{\partial F}{\partial v}$ are bounded, $U, V$ are continuous with respect to $\mu$, uniformly continuous to $u$ and $v$ in the region $r_1^2 \leqslant u^2+v^2 \leqslant r_0^2$, there exists a sufficiently small $\mu_0 > 0$ such that for $0 \leqslant \mu \leqslant \mu_0$, the second term of the right-hand side of (14) is less than $\frac{C_0}{2} r_1^{2k}$. Thus, in the annular region $r_1^2 \leqslant u^2+v^2 \leqslant r_0^2$, for $0 \leqslant \mu \leqslant \mu_0$,

$$\left.\frac{\mathrm{d}F}{\mathrm{d}t}\right|_{(12)-\mu} < 0.$$

Therefore, when the parameter $\mu$ satisfies $0 \leqslant \mu \leqslant \mu_0$, the trajectory of $(12)-\mu$ crosses the curve $F(u,v) = a_0$ from the outside to inside. For $\mu > 0$, only unstable focus can be in the region $\Omega$, where $\Omega$ is the region bounded by the curve $F(u,v) = a_0$. The Poincare–Bendixson theorem implies that there exists at least a limit cycle which is stable in $\Omega$. Moreover, when the boundary of $\Omega$: $F(x_1,x_2) = a_0$ shrinks to the origin, $r_1 \to 0$, $\mu_0 \to 0$. For sufficiently small $\mu$, there exists a stable limit cycle around the origin of system $(11)-\mu$. Assume the equations of the limit cycle are $x_1 = \phi(t, \mu), x_2\psi(t, \mu)$, then, system $(7) - \mu$ has the following asymptotically stable closed orbit in the surface $M_\mu$:

$$\Gamma_\mu : x_1 = \phi(t, \mu), \qquad x_2 = \psi(t, \mu), \qquad x_3 = \chi(t, \mu) \equiv x_3 (\phi(t, \mu), \psi(t, \mu), \mu),$$

which has the same period as the one in system $(11) - \mu$.

We would like to point it out that the asymptotically stable closed orbit in the surface $M_\mu$ is also asymptotically stable is the whole space. To that purpose, we need to check the three eigenvalues of the image $D\varphi_{T_\mu}(\bar{X}, \mu)$, where $\bar{X} \in \Gamma_\mu$, and $T_\mu$ is the period of $\Gamma_\mu$. Since when $\mu$ is sufficiently small, the distance between the images $D\varphi_{T_\mu}(\bar{X}, \mu)$ and $D\varphi_{T_0}(O, 0)$ could be as small as possible, thus the image $D\varphi_{T_\mu}(\bar{X}, \mu)$ has an eigenvalue whose eigenvector is not tangent to $M_\mu$, which could be as close as possible to the eigenvalue $e^{2\pi\delta(0)/\beta(0)}$ $(< 1)$ of $D\varphi_{T_0}(O, 0)$. Moreover, for the other two eigenvalues of $D\varphi_{T_\mu}(\bar{X}, \mu)$, the one along with the closed orbit is always 1, and the other must be $\leqslant 1$ since $\Gamma_\mu$ is asymptotically stable in $M_\mu$. If it is less than 1, then the image $D\varphi_{T_\mu}(\bar{X}, \mu)$ has two eigenvalues whose absolute value is less than 1. (In general, in $R^n$ if there exists a point $P$ in a close orbit $\gamma$ such that the corresponding linear image $D\varphi(P)$ has $n-1$ eigenvalues whose absolute value is less than 1, then $\gamma$ is asymptotically stable.)

If the eigenvalue is 1, then $\Gamma_\mu$ is the limit cycle with a zero index, a critical case, which can be analyzed by the local attraction property of the center manifold to show the asymptotical stability of $\Gamma_\mu$ in the space.

Finally, we would like to mention if the derived operator $Df(O, \wp) = \wp(\mu)$ on the right-hand side function of $(7){-}\mu$ at the origin does not have the standard form (8), make a coordinate change by using the eigenvectors. Since the right-hand side function of $(7){-}\mu$ has a parameter $\mu$, in order to make sure that the function is still analytic of $X$ and $\mu$, the eigenvectors need to be the functions of $\mu$. In fact, suppose $\xi_k$ is the corresponding eigenvector of the $k$th eigenvalue $a_k$ of the matrix $\wp$. By the Cauchy formula of the matrix function, we can write the projecting operator $P_k$ onto the $k$th eigenvector $\xi_k$ as

$$P_k = \frac{1}{2\pi i} \oint_{\Gamma_k} (\lambda I - \wp)^{-1} \mathrm{d}\lambda,$$

where, $\Gamma_k$ is a closed curve surrounding $a_k$ only. (Note, that $\Gamma_k$ does not surround $a_{j,}(j \neq k)$). Therefore, if $\wp = \wp(\mu)$ is an analytic function of $\mu$, so are its eigenvectors. We thus complete the proof of lemma 1.

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