# Analysis of a mathematical model of syntrophic bacteria in a chemostat

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# Analysis of a mathematical model of syntrophic bacteria in a chemostat

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**Abstract** A mathematical model involving a syntrophic relationship between two populations of bacteria in a continuous culture is proposed. A detailed qualitative analysis is carried out. The local and global stability analysis of the equilibria are performed. We demonstrate, under general assumptions of monotonicity, relevant from an applied point of view, the asymptotic stability of the positive equilibrium point which corresponds to the coexistence of the two bacteria. A syntrophic relationship in the anaerobic digestion process is proposed as a real candidate for this model.

**Keywords** Syntrophic relationship · Mathematical modelling · Coexistence · Asymptotic stability · Anaerobic digestion

## 1 Introduction

A synthrophic relationship between two organisms refers to a situation where the species exhibit mutualistism but where, at the opposite of what happens in a purely symbiotic relationship, one of the species can grow without the other. Such a situation can be mathematically formalized as follows. Assume that a first species denoted $X_1$ grows on a substrate $S_1$ forming an intermediate product $S_2$. This intermediate product is required by a second species $X_2$ to grow. The limiting substrate of the second bacteria being the product of the first bioreaction, the second bacteria cannot grow if the first one is not present.

Such interactions are quite common in nature: it is why a number of models have already been proposed in the literature. In Katsuyama *et al.*, a model involving two mutualistic species is proposed for describing pesticide degradation, cf. [9] while a more general case is considered in [10]. Since mutualism involves generally species interacting through intermediate products, other studies consider mutualistic relationships in food

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webs. For instance, Bratbak and Thingstad, or more recently, Aota and Nakajima considered the mutualism between phytoplankton and bacteria through the carbon excretion by the phytoplankton, cf. [2], [1]. A model studied by Freedman *et al.* was proposed to explain the observed coexistence of such species. However, in the previous studies the models are very specific. In particular, the mathematical analyses of the models are realized for specific growth rates that are explicitely given (in most cases as Monod functions).

To extend the study of mutualism to more general systems, we have recently considered more general assumptions notably with respect to the growth rate functions considered in the models in using qualitative hypotheses, cf. [5] . Furthermore, it was assumed that the species $X_1$ may be inhibited by the product $S_2$ that it produces itself while the species $X_2$ may be inhibited by an excess of substrate $S_1$. An example of such interactions is given by the anaerobic digestion in which mutualistic relationships allow certain classes of bacteria to coexist.

In the actual paper, we revisit the model proposed in [5] in considering two main changes which significantly further extend the range of practical situations covered by the model. First, we assume that there is some $S_2$ in the influent. In other terms, the limiting substrate $S_2$ on which the species $X_2$ grows is not only produced by the species $X_1$ but is also available even if the species $X_1$ is not present. The second modification of the model is that the second species is supposed to be inhibited by an excess of $S_1$, the limiting substrate on which the first species grows. To illustrate the usefulness of such extensions of the original model by El Hajji et al. [5], the biological interpretation of these hypotheses within the context of the anaerobic process is given in the appendix.

The paper is organized as follows. In section 2, we propose a modified system of four differential equations from the original model in [5]. The positive equilibria are determined and their local and global stability properties are established. The global asymptotic stability results are demonstrated through the index of Poincaré proving the uniqueness of the positive equilibrium point, the Dulac's criterion that rules out the possibility of the existence of periodic solutions for the reduced planar system, the Poincaré-Bendixon Theorem and the Butler-McGehee Lemma. In particular, we show that for every positive initial conditions, and under general and natural assumptions on the substrate input concentration $S_1^{in}$, the intermediate product input concentration $S_2^{in}$ and on the dilution rate $D$, the solutions converge to the positive equilibrium point which corresponds to the coexistence of the two bacterial species as observed in real processes. Simulations are presented in Section 3, an example of a syntrophic relationship is given in section 4 as a candidate for this model while concluding remarks are given in Section 5.

## 2 Mathematical model and results

### 2.1 Mathematical model

Let $S_1$, $X_1$, $S_2$ and $X_2$ denote, respectively, the concentrations of the substrate, the first bacteria, the intermediate product, and the second bacteria present in the reactor at time $t$. We neglect all species-specific death rates and take into account the dilution rate only. Hence our model is described by the following system of ordinary differential equations :

$$\begin{cases} \dot{S}_1 = D(S_1^{in} - S_1) - k_3\mu_1(S_1,S_2)X_1 \ , \\ \dot{X}_1 = \mu_1(S_1,S_2)X_1 - DX_1 \ , \\ \dot{S}_2 = D(S_2^{in} - S_2) - k_2\mu_2(S_1,S_2)X_2 + k_1\mu_1(S_1,S_2)X_1 \ , \\ \dot{X}_2 = \mu_2(S_1,S_2)X_2 - DX_2 \ . \end{cases} \tag{1}$$

Where $S_1^{in} > 0$ denotes the input concentration of substrate, $S_2^{in} > 0$ denotes the input concentration of the intermediate product and $D > 0$ is the dilution rate.

The functional response of each species $\mu_1, \mu_2 : \mathbb{R}^2_+ \to \mathbb{R}_+$ satisfies :
*A1.* $\mu_1, \mu_2 : \mathbb{R}^2_+ \to \mathbb{R}_+$, of class $\mathscr{C}^1$ ,
*A2.* $\mu_1(0,S_2) = 0, \qquad \mu_2(S_1,0) = 0, \quad \forall\, (S_1,S_2) \in \mathbb{R}^2_+$ ,
*A3.* $\dfrac{\partial \mu_1}{\partial S_1}(S_1,S_2) > 0, \quad \dfrac{\partial \mu_1}{\partial S_2}(S_1,S_2) < 0, \quad \forall\, (S_1,S_2) \in \mathbb{R}^2_+$ ,
*A4.* $\dfrac{\partial \mu_2}{\partial S_1}(S_1,S_2) < 0, \quad \dfrac{\partial \mu_2}{\partial S_2}(S_1,S_2) > 0, \quad \forall\, (S_1,S_2) \in \mathbb{R}^2_+$ .

Hypothesis *A2* expresses that no growth can take place for species $X_1$ without the substrate $S_1$ and that the intermediate product $S_2$ is obligate for the growth of species $X_2$. Hypothesis *A3* means that the growth of species $X_1$ increases with the substrate $S_1$ and it is inhibited by the intermediate product $S_2$ that it produces. Hypothesis *A4* means that the growth of species $X_2$ increases with intermediate product $S_2$ produced by species $X_1$ while it is inhibited by the substrate $S_1$. Note that there is a syntrophic relationship between the two species.

We first scale system (1) using the following change of variables and notations :

$$s_1 = \frac{k_1}{k_3} S_1, \quad x_1 = k_1 X_1, \quad s_2 = S_2, \quad x_2 = k_2 X_2, \quad s_1^{in} = \frac{k_1}{k_3} S_1^{in}, \quad s_2^{in} = S_2^{in} .$$

The dimensionless equations thus obtained are :

$$\begin{cases} \dot{s}_1 = D(s_1^{in} - s_1) - f_1(s_1,s_2)x_1 , \\ \dot{x}_1 = f_1(s_1,s_2)x_1 - Dx_1 , \\ \dot{s}_2 = D(s_2^{in} - s_2) - f_2(s_1,s_2)x_2 + f_1(s_1,s_2)x_1 , \\ \dot{x}_2 = f_2(s_1,s_2)x_2 - Dx_2 . \end{cases} \tag{2}$$

Where the functions $f_1, f_2 : \mathbb{R}^2_+ \to \mathbb{R}_+$ are defined by

$$f_1(s_1,s_2) = \mu_1(\frac{k_3}{k_1}s_1, s_2) \quad \text{and} \quad f_2(s_1,s_2) = \mu_2(\frac{k_3}{k_1}s_1, s_2).$$

These functions satisfy the following assumptions :
*H1.* $f_1, f_2 : \mathbb{R}^2_+ \to \mathbb{R}_+$, of class $\mathscr{C}^1$ ,
*H2.* $f_1(0,s_2) = 0, \qquad f_2(s_1,0) = 0, \quad \forall\, (s_1,s_2) \in \mathbb{R}^2_+$ ,
*H3.* $\dfrac{\partial f_1}{\partial s_1}(s_1,s_2) > 0, \quad \dfrac{\partial f_1}{\partial s_2}(s_1,s_2) < 0, \quad \forall\, (s_1,s_2) \in \mathbb{R}^2_+$ ,
*H4.* $\dfrac{\partial f_2}{\partial s_1}(s_1,s_2) < 0, \quad \dfrac{\partial f_2}{\partial s_2}(s_1,s_2) > 0, \quad \forall\, (s_1,s_2) \in \mathbb{R}^2_+$ .

It is easy to see that $\mathbb{R}^4_+$, the closed non-negative cone in $\mathbb{R}^4$, is positively invariant under the solution map of system (2). More precisely

**Proposition 1** *For every initial condition in $\mathbb{R}^4_+$, the solution of system (2) has positive components and is positively bounded and thus is defined for every positive $t$. The set*

$$\Omega = \left\{ (s_1,x_1,s_2,x_2) \in \mathbb{R}^4_+ : \; s_1 + x_1 = s_1^{in}, \quad s_2 + x_2 = x_1 + s_2^{in} \right\}$$

*is a positive invariant attractor of all solutions of system (2).*

**Proof:** The invariance of $\mathbb{R}^4_+$ is guaranteed by the fact that :

i. $s_1 = 0 \Rightarrow \dot{s}_1 = D\, s_1^{in} > 0$,
ii. $s_2 = 0 \Rightarrow \dot{s}_2 = D\, s_2^{in} + f_1(s_1,0)\, x_1 > 0$,

iii. $x_i = 0 \Rightarrow \dot{x}_i = 0$ for $i = 1,2$.

Next we have to prove that the solution is bounded. Let $z_1 = s_1 + x_1$, then $\dot{z}_1 = -D(z_1 - s_1^{in})$ from which one deduces :

$$s_1(t) + x_1(t) = s_1^{in} + (s_1(0) + x_1(0) - s_1^{in})e^{-Dt} \ . \tag{3}$$

Thus $s_1(t)$ and $x_1(t)$ are positively bounded. Let $z_2 = s_2 + x_2 - x_1$, then $\dot{z}_2 = -D(z_2 - s_2^{in})$ from which one deduces:

$$s_2(t) + x_2(t) - x_1(t) = s_2^{in} + (s_2(0) + x_2(0) - x_1(0) - s_2^{in})e^{-Dt} \ . \tag{4}$$

Thus $s_2(t)$ and $x_2(t)$ are positively bounded. Hence, the solution is defined for all positive $t$. From (3) and (4) we deduce that the set $\Omega$ is an invariant set which is an attractor. ■

## 2.2 Restriction on the plane

The solutions of system (2) are exponentially convergent towards the set $\Omega$ and we are interested in the asymptotic behavior of these solutions. It is enough to restrict the study of the asymptotic behaviour of system (2) to $\Omega$. In fact, thanks to Thieme's results [12], the asymptotic behaviour of the solutions of the restriction of (2) on $\Omega$ will be informative for the complete system, see Section 2.5. In this section we study the following reduced system which is simply the projection on the plane $(x_1, x_2)$, of the restriction of system (2) on $\Omega$.

$$\begin{cases} \dot{x}_1 = \left[ f_1\left(s_1^{in} - x_1, s_2^{in} + x_1 - x_2\right) - D \right] x_1 \ , \\ \dot{x}_2 = \left[ f_2\left(s_1^{in} - x_1, s_2^{in} + x_1 - x_2\right) - D \right] x_2 \ . \end{cases} \tag{5}$$

Thus, for (5) the state-vector$(x_1, x_2)$ belongs to the following subset of the plane, see Fig. 1 :

$$\mathscr{S} = \left\{ (x_1, x_2) \in {\mathbb{R}_+}^2 : 0 < x_1 \leq s_1^{in}, 0 < x_2 \leq x_1 + s_2^{in} \right\}.$$

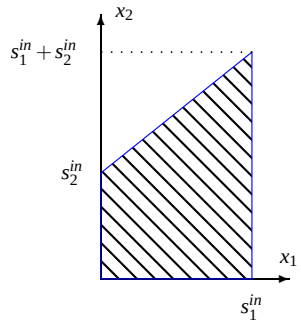


**Fig. 1** The set $\mathscr{S}$

We study the equilibria of system (5) which we label as

$$F^0 = (0,0), \quad F^1 = (\bar{x_1}, 0), \quad F^2 = (0, \tilde{x_2}), \quad \text{and} \quad F^* = (x_1^*, x_2^*).$$

Notice that $F^0, F^1, F^2$ and $F^*$ are the project of the equilibrium points $E^0 = (s_1^{in}, 0, s_2^{in}, 0), E^1 = (\bar{s_1}, \bar{x_1}, s_2^{in}, 0), E^2 = (s_1^{in}, 0, \tilde{s_2}, \tilde{x_2})$ and $E^* = (s_1^*, x_1^*, s_2^*, x_2^*)$ of system (2) on the plane $(x_1, x_2)$.
Let $D_1 = f_1(s_1^{in}, s_2^{in}), D_2 = f_2(s_1^{in} - \bar{x_1}, s_2^{in} + \bar{x_1}), D_3 = f_1(s_1^{in}, s_2^{in} - \tilde{x_2})$ and $D_4 = f_2(s_1^{in}, s_2^{in})$. Notice that $D_1 < D_3$ and $D_4 < D_2$.

The trivial equilibrium point $F^0$ always exists. It nature is given in the following lemma.

**Lemma 1** *If $D > \max(D_1, D_4)$ then $F^0$ is a stable node. If $\min(D_1, D_4) < D < \max(D_1, D_4)$ then $F^0$ is a saddle point. If $D < \min(D_1, D_4)$ then $F^0$ is an unstable node.*

**Proof.** The Jacobian matrix, $J$, at a point $(x_1, x_2)$ is given by :

$$J = \begin{bmatrix} -\dfrac{\partial f_1}{\partial s_1}x_1 + \dfrac{\partial f_1}{\partial s_2}x_1 + f_1 - D & \dfrac{\partial f_1}{\partial s_2}x_1 \\ -\dfrac{\partial f_2}{\partial s_1}x_2 + \dfrac{\partial f_2}{\partial s_2}x_2 & -\dfrac{\partial f_2}{\partial s_2}x_2 + f_2 - D \end{bmatrix}.$$

The Jacobian matrix at $F^0$ is given by :

$$J^0 = \begin{bmatrix} f_1(s_1^{in}, s_2^{in}) - D & 0 \\ 0 & f_2(s_1^{in}, s_2^{in}) - D \end{bmatrix}$$

The eigenvalues are $D_1 - D$ and $D_4 - D$. Thus, if $D > \max(D_1, D_4)$ then $F^0$ is a stable node. It is an unstable node if $D < \min(D_1, D_4)$. It is a saddle point if $\min(D_1, D_4) < D < \max(D_1, D_4)$. ■

The conditions of existence of the boundary equilibria $F^1$ and $F^2$ are stated in the following lemmas.

**Lemma 2** *An equilibrium $F^1$ exists if and only if $D < D_1$. If it exists then it the unique equilibrium on the positive $x_1$ semi-axis. It is a saddle point if $D < D_2$, and a stable node if $D > D_2$.*

**Proof.** An equilibrium $F^1$ exists if and only if $\bar{x_1} \in ]0, s_1^{in}[$ is a solution of

$$f_1(s_1^{in} - \bar{x_1}, s_2^{in} + \bar{x_1}) = D. \tag{6}$$

Let $\psi_1(x_1) = f_1(s_1^{in} - x_1, s_2^{in} + x_1) - D$. Since $\psi_1'(x_1) < 0,\ \psi_1(0) = D_1 - D,\ \psi_1(s_1^{in}) = -D < 0$, equation (6) admits a positive solution if and only if $D < D_1$. If this condition is satisfied then (6) admits a unique solution since the function $\psi_1(.)$ is decreasing.
The Jacobian matrix at $F^1$ is given by :

$$J^1 = \begin{bmatrix} -\dfrac{\partial f_1}{\partial s_1}\bar{x_1} + \dfrac{\partial f_1}{\partial s_2}\bar{x_1} & \dfrac{\partial f_1}{\partial s_2}\bar{x_1} \\ 0 & f_2 - D \end{bmatrix}$$

where the functions are evaluated at $(s_1^{in} - \bar{x_1}, s_2^{in} + \bar{x_1})$. The eigenvalues are $D_2 - D$ and $-\dfrac{\partial f_1}{\partial s_1}\bar{x_1} + \dfrac{\partial f_1}{\partial s_2}\bar{x_1} < 0$. Thus $F^1$ is a saddle point if $D > D_2$. It is a stable node if the inequality is reversed. ■

**Lemma 3** *An equilibrium $F^2$ exists if and only if $D < D_4$. If it exists then it the unique equilibrium on the positive $x_2$ semi-axis. If it exists then it is a saddle point if $D < D_3$, and a stable node if $D > D_3$.*

**Proof.** An equilibrium $F^2$ exists if and only if $\tilde{x}_2 \in ]0, s_2^{in}[$ is a solution of

$$f_2(s_1^{in}, s_2^{in} - \tilde{x}_2) = D. \tag{7}$$

Let $\psi_2(x_2) = f_2(s_1^{in}, s_2^{in} - x_2) - D$. Since $\psi_2'(x_2) < 0,\ \psi_2(0) = D_4 - D,\ \psi_2(s_2^{in}) = -D < 0$, equation (7) admits a positive solution if and only if $D < D_4$. If this condition is satisfied then (7) admits a unique solution since the function $\psi_2(.)$ is decreasing. The Jacobian matrix at $F^2$ is given by :

$$J^2 = \begin{bmatrix} f_1 - D & 0 \\ -\dfrac{\partial f_2}{\partial s_1}\tilde{x}_2 + \dfrac{\partial f_2}{\partial s_2}\tilde{x}_2 & -\dfrac{\partial f_2}{\partial s_2}\tilde{x}_2 \end{bmatrix}$$

where the functions are evaluated at $(s_1^{in}, s_2^{in} - \tilde{x}_2)$. The eigenvalues are $D_3 - D$ and $-\frac{\partial f_2}{\partial s_2}\tilde{x}_2 < 0$. Thus $F^2$ is a saddle point if $D < D_3$. It is a stable node if the inequality is reversed. ■

The nature of equilibrium $F^*$ is given in the following lemma

**Lemma 4** *If an equilibrium $F^*$ exists then it is exponentially asymptotically stable.*

**Proof.** The Jacobian matrix at $F^*$ is given by :

$$J^* = \begin{bmatrix} -2\frac{\partial f_1}{\partial s_1}x_1^* + \frac{\partial f_1}{\partial s_2}x_1^* & \frac{\partial f_1}{\partial s_2}x_1^* \\ -2\frac{\partial f_2}{\partial s_1}x_2^* + \frac{\partial f_2}{\partial s_2}x_2^* & -\frac{\partial f_2}{\partial s_2}x_2^* \end{bmatrix}$$

Notice that

$$\det(J^*) = -\frac{\partial f_2}{\partial s_2}x_2^*\left(-2\frac{\partial f_1}{\partial s_1}x_1^* + \frac{\partial f_1}{\partial s_2}x_1^*\right) - \frac{\partial f_1}{\partial s_2}x_1^*\left(-2\frac{\partial f_2}{\partial s_1}x_2^* + \frac{\partial f_2}{\partial s_2}x_2^*\right) > 0$$

and

$$\mathrm{tr}(J^*) = -2\frac{\partial f_1}{\partial s_1}x_1^* + \frac{\partial f_1}{\partial s_2}x_1^* - \frac{\partial f_2}{\partial s_2}x_2^* < 0$$

then $J^*$ admits two eigenvalues with negative real part. Then, if it exists, $F^*$ is exponentially stable. ■

Concerning positive equilibria, notice that an equilibrium $F^*$ exists if and only if $(x_1^*, x_2^*) \in \mathscr{S}$ is a solution of

$$f_1(s_1^{in} - x_1^*, s_2^{in} + x_1^* - x_2^*) = f_2(s_1^{in} - x_1^*, s_2^{in} + x_1^* - x_2^*) = D\,.$$

It is not easy to discuss the number of solutions of this set of equations. In the next section we will use the index theory to obtain the number of positive equilibria $F^*$. Notice that if $F^*$ and $F^1$ exist then

$$f_1(s_1^{in} - x_1^*, s_2^{in} + x_1^*) < f_1(s_1^{in} - x_1^*, s_2^{in} + x_1^* - x_2^*) = D = f_1(s_1^{in} - \bar{x_1}, s_2^{in} + \bar{x_1}).$$

Hence $\psi_1(x_1^*) < \psi_1(\bar{x_1})$, from where one obtains $0 < \bar{x_1} < x_1^* < s_1^{in}$. If $F^*$ and $F^2$ exist then

$$f_2(s_1^{in}, s_2^{in} - x_2^*) < f_2(s_1^{in} - x_1^*, s_2^{in} + x_1^* - x_2^*) = D = f_2(s_1^{in}, s_2^{in} - \tilde{x}_2).$$

Hence $\psi_2(x_2^*) < \psi_2(\tilde{x}_2)$, from where one obtains $0 < \tilde{x}_2 < x_2^* < s_2^{in} + s_1^{in}$.

### 2.3 Main result

The number of equilibria of (5) and their nature are summarized in the next Theorem.

**Theorem 1**

1. *If $D < \min(D_1, D_4)$ then (5) admits four equilibria : $F^0$ which is an unstable node, $F^1$ and $F^2$ which are saddle points and $F^*$ which is a stable node.*
2. *If $\min(D_1, D_4) < D < \max(D_1, D_4)$, four subcases must be distinguished*
   - *If $D_1 < D_4$ and $D_1 < D < \min(D_3, D_4)$ then (5) admits three equilibria : $F^0$ and $F^2$ which are saddle points and $F^*$ which is a stable node.*
   - *If $D_1 < D_4$ and $D_3 < D < D_4$ then (5) admits two equilibria : $F^0$ which is a saddle point and $F^2$ which is a stable node.*
   - *If $D_4 < D_1$ and $D_4 < D < \min(D_1, D_2)$ then (5) admits three equilibria : $F^0$ and $F^1$ which are saddle points and $F^*$ which is a stable node.*

- *If $D_4 < D_1$ and $D_2 < D < D_1$ then (5) admits two equilibria : $F^0$ which is a saddle point and $F^1$ which is a stable node.*

3. *If $D > \max(D_1, D_4)$ then (5) admits one equilibrium : $F^0$ which is a stable node.*

**Proof.** We must show that the positive equilibrium $F^*$ is unique when it exists. For this purpose we use the index theory. Let $\delta$ be a small enough positive constant. Let

$$\mathscr{S}_\delta = \left\{(x_1,x_2) \in \mathbb{R}^2 \ : \ -\delta < x_1 \leq s_1^{in} \quad \text{and} -\delta < x_2 \leq x_1 + s_2^{in}\right\}.$$

Consider the vector field $G$ on $\mathscr{S}_\delta$ defined as follows :

$$G(x_1,x_2) = \begin{pmatrix} (\tilde{f}_1(s_1^{in} - x_1, s_2^{in} + x_1 \ - x_2) - D)x_1 \\ \\ (\tilde{f}_2(s_1^{in} - x_1, s_2^{in} + x_1 \ - x_2) - D)x_2 \end{pmatrix}$$

where $\tilde{f}_1$ and $\tilde{f}_2$ are two $\mathscr{C}^1$ functions prolonging respectively functions $f_1$ and $f_2$ on $\mathscr{S}_\delta$. Assume that the vector field $G$ has $n$ singular points $F_i^*$, $i = 1 \cdots n$.

1. Assume that $D < \min(D_1, D_4)$. From the previous lemmas the system has three singular points $F^0, F^1$ and $F^2$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta) \cup \mathscr{C}(F^1, \delta) \cup \mathscr{C}(F^2, \delta)$ which surrounds all singular points of the vector field $G$. The winding number of $G$ on $J$ equals the sum of the indexes of these singular points. Since $F^0$ is an unstable node, of index 1, $F^1$ and $F^2$ are saddle points, of index -1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to 0, see Fig. 3 (a). Thus $1 - 2 + n = 0$, which gives $n = 1$, from where the positive equilibrium $F^*$ exists and is unique.
2. Assume that $\min(D_1, D_4) < D < \max(D_1, D_4)$
   - If $D_1 < D_4$ and $D_1 < D < \min(D_3, D_4)$. From the previous lemmas the system has two singular points $F^0$ and $F^2$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta) \cup \mathscr{C}(F^2, \delta)$ which surrounds all singular points of the vector field $G$. Since $F^0$ and $F^2$ are saddle points, of index -1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to -1, see Fig. 3 (b). Thus $-2 + n = -1$, which gives $n = 1$, from where the positive equilibrium $F^*$ exists and is unique.
   - If $D_1 < D_4$ and $D_3 < D < D_4$. From the previous lemmas the system has two singular points $F^0$ and $F^2$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta) \cup \mathscr{C}(F^2, \delta)$ which surrounds all singular points of the vector field $G$. Since $F^0$ is a saddle point, of index -1, $F^2$ is a stable node, of index 1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to 0, see Fig. 3 (c). Thus $1 - 1 + n = 0$, which gives $n = 0$, from where there is no positive equilibrium points $F^*$.
   - If $D_4 < D_1$ and $D_4 < D < \min(D_1, D_2)$. From the previous lemmas the system has two singular points $F^0$ and $F^1$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta) \cup \mathscr{C}(F^1, \delta)$ which surrounds all singular points of the vector field $G$. Since $F^0$ and $F^1$ are saddle points, of index -1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to -1, see Fig. 3 (d). Thus $-2 + n = -1$, which gives $n = 1$, from where the positive equilibrium $F^*$ exists and is unique.
   - If $D_4 < D_1$ and $D_2 < D < D_1$. From the previous lemmas the system has two singular points $F^0$ and $F^1$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta) \cup \mathscr{C}(F^1, \delta)$ which surrounds all singular points of the vector field $G$. Since $F^0$ is a saddle point, of index -1, $F^1$ is stable node, of index 1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to 0, see Fig. 3 (e). Thus $1 - 1 + n = 0$, which gives $n = 0$, from where there is no positive equilibrium points $F^*$.

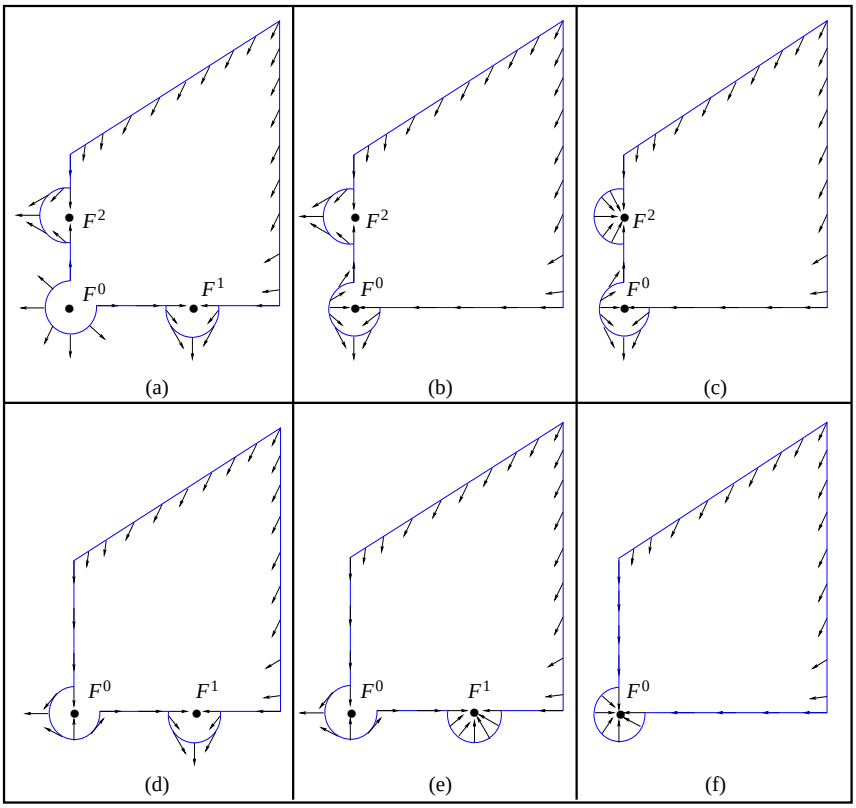


**Fig. 2** The winding number of $G$ on $J$

3. Assume that $D > \max(D_1, D_4)$. From the previous lemmas the system has a singular point $F^0$ and possibly $n$ equilibria $F_i^*$, $i = 1 \cdots n$. Define the Jordan curve $J = \partial\mathscr{S} \cup \mathscr{C}(F^0, \delta)$ which surrounds all singular points of the vector field $G$. Since $F^0$ is a stable node, of index 1 and $F_i^*$, $i = 1 \cdots n$, are stable nodes, of index 1. The winding number of the vector field $G$ on the closed curve $J$ is equal to 1, see Fig. 3 (f). Thus $1 + n = 1$, which gives $n = 0$, from where there is no positive equilibrium points $F^*$. ■

As it is shown in [7], when $D < \min(D_1, D_4)$, the theorem can be proved through an approach using the well-known Poincaré-Hopf index Theorem recalled hereafter.

**Theorem 2** *Let $M$ be a compact orientable differentiable manifold. Let $v$ be a vector field on $M$ with isolated zeroes. If $M$ has boundary, then $v$ must be pointing in the inward normal direction along the boundary. Then we have the formula*

$$\Sigma_i \Big( index_v(p_i) \Big) = \chi(M)$$

*where the sum of the indices is over all the isolated zeroes of $v$ and $\chi(M)$ is the Euler characteristic of $M$.*

We identify the segment $[0, s_1^{in}]$ of the $x$-axis with the segment $[0, s_2^{in}]$ of the $y$-axis such the saddle point $F^1 = (\bar{x_1}, 0)$ corresponds to the saddle point $F^2 = (0, \tilde{x}_2)$ using the following piecewise affine function :

$$x_2 = \begin{cases} \dfrac{\tilde{x}_2}{\bar{x_1}} x_1 & \text{if} \quad x_1 \in (0, \bar{x_1}), \\ \dfrac{x_1 - \bar{x_1}}{s_1^{in} - \bar{x_1}} (s_2^{in} - \tilde{x}_2) + \tilde{x}_2 & \text{if} \quad x_1 \in (\bar{x_1}, s_1^{in}). \end{cases}$$

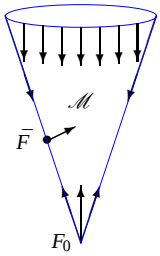


**Fig. 3** The cone $\mathscr{M}$

The geometry is changed and one obtains a cone $\mathscr{M}$ as image of $\mathscr{S}$ (see Fig. 3) with a vector field having one source (the origin $p_1 = F_0$), one saddle point ($p_2 = \bar{F}$; the result of the identifying of the two saddle points $F^1$ and $F^2$) and $n$ sinks ($p_i, i = 3, n+2$). The Euler-Poincaré characteristic of $\mathscr{M}$ is equal to 1. Using the Poincaré-Hopf index Theorem one has $n+1+(-1) = 1$, then $n = 1$ from where the positive equilibrium point $F^*$ exists and is unique.
The same proof will work in the case when $D > \min(D_1, D_4)$. In this case we simply identify the segment $[0, s_1^{in}]$ of the $x$-axis with the segment $[0, s_2^{in}]$ of the $y$-axis using the affine function $x_2 = \frac{s_2^{in}}{s_1^{in}} x_1$. We obtain a cone $\mathscr{M}$ as image of $\mathscr{S}$ with a vector field having one sink (the origin $F_0$) and $n$ sinks. Using the Poincaré-Hopf index Theorem one has $n+1 = 1$, then $n = 0$ from where the positive equilibrium point $F^*$ does not exist.

## 2.4 Global analysis of the 2D system

Let us establish first that (5) admits no periodic orbit nor polycycle inside $\mathscr{S}$

**Theorem 3** *There are no periodic orbits nor polycycles inside $\mathscr{S}$.*

**Proof.** Consider a trajectory of (5) belonging to $\mathscr{S}$. Let us transform the system (5) through the change of variables $\xi_1 = \ln(x_1)$, $\xi_2 = \ln(x_2)$. Then one obtains the following system :

$$\begin{cases} \dot{\xi_1} = h_1(\xi_1, \xi_2) := f_1(s_1^{in} - e^{\xi_1}, s_2^{in} + e^{\xi_1} - e^{\xi_2}) - D, \\ \dot{\xi_2} = h_1(\xi_1, \xi_2) := f_2(s_1^{in} - e^{\xi_1}, s_2^{in} + e^{\xi_1} - e^{\xi_2}) - D. \end{cases} \tag{8}$$

We have

$$\frac{\partial h_1}{\partial \xi_1} + \frac{\partial h_2}{\partial \xi_2} = -e^{\xi_1} \frac{\partial f_1}{\partial s_1} + e^{\xi_1} \frac{\partial f_1}{\partial s_2} - e^{\xi_2} \frac{\partial f_2}{\partial s_2} < 0.$$

From Dulac criterion [11], we deduce that the system (8) has no periodic trajectory. Hence (5) has no periodic orbit in $\mathscr{S}$. ■

**Theorem 4** *For every initial condition in $\mathscr{S}$, the trajectories of system (5) converge asymptotically to :*

- $F^*$ *if* $D < \min(D_1, D_4)$.
- $F^*$ *if* $D_1 < D_4$ *and* $D_1 < D < \min(D_3, D_4)$.
- $F^*$ *if* $D_4 < D_1$ *and* $D_4 < D < \min(D_1, D_2)$.
- $F^2$ *if* $D_1 < D_4$ *and* $D_3 < D < D_4$.
- $F^1$ *if* $D_4 < D_1$ *and* $D_2 < D < D_1$.
- $F^0$ *if* $\max(D_1, D_4) < D$.

**Proof :** We restrict the proof to the situation where $D < \min(D_1, D_4)$. The other cases can be done similarly. Let $x_1(0) > 0, x_2(0) > 0$ and $\omega$ the $\omega$-limit set of $(x_1(0), x_2(0))$. $\omega$ is an invariant compact set and $\omega \subset \mathscr{S}$. Assume that $\omega$ contains a point $M$ on the $x_1x_2$ axis :

- $M$ can't be $F^0$ because $F^0$ is an unstable node and can't be a part of the $\omega$-limit set of $(x_1(0), x_2(0))$,
- If $M \in ]\bar{x_1}, s_1^{in}] \times \{0\}$ (respectively $M \in \{0\} \times ]\bar{x_2}, s_2^{in}]$). As $\omega$ is invariant then $\gamma(M) \subset \omega$ which is impossible because $\omega$ is bounded and $\gamma(M) = ]\bar{x_1}, +\infty[ \times \{0\}$ (respectively $\gamma(M) = \{0\} \times ]\bar{x_2}, +\infty[$),
- If $M \in ]0, \bar{x_1}[ \times \{0\}$ (respectively $M \in \{0\}\ , \times ]0, \bar{x_2}[$). $\omega$ contains $\gamma(M) = ]0, \bar{x_1}[ \times \{0\}$ (respectively $\gamma(M) = \{0\} \times ]0, \bar{x_2}[$). As $\omega$ is a compact, then it contains the adherence of $\gamma(M)$, $[0, \bar{x_1}] \times \{0\}$ (respectively $\{0\} \times [0, \bar{x_2}]$). In particular, $\omega$ contains $F^0$ which is impossible,
- If $M = F^1$ (respectively $M = F^2$). $\omega$ is not reduced to $F^1$ (respectively to $F^2$). By Butler-McGehee theorem, $\omega$ contains a point $P$ of $(0, +\infty) \times \{0\}$ other that $F^1$ (respectively of $\{0\} \times (0, +\infty)$ other that $F^2$) which is impossible.

Finally, the $\omega$-limit set don't contain any point on the $x_1x_2$ axis. System (5) has no periodic orbit inside $\mathscr{S}$. Using the Poincaré-Bendixon Theorem [11], $F^*$ is a globally asymptotically stable equilibrium point for system (5). ■

**Remark 1** *One can also used the fact that the system (5) has no periodic orbit inside $\mathscr{S}$. Sight nature of $F^0, F^1$ and $F^2$, the system (5) can't have a cyclique chain on $\partial\mathscr{S}$. Using the Poincaré-Bendixon Theorem [11], $F^*$ is a globally asymptotically stable equilibrium point for system (5).*

### 2.5 Global analysis of the 4D system

**Theorem 5** *For every initial condition in $\mathbb{R}^4_+$, the trajectories of system (2) converge asymptotically to :*

- $E^*$ *if* $D < \min(D_1, D_4)$.
- $E^*$ *if* $D_1 < D_4$ *and* $D_1 < D < \min(D_3, D_4)$.
- $E^*$ *if* $D_4 < D_1$ *and* $D_4 < D < \min(D_1, D_2)$.
- $E^2$ *if* $D_1 < D_4$ *and* $D_3 < D < D_4$.
- $E^1$ *if* $D_4 < D_1$ *and* $D_2 < D < D_1$.
- $E^0$ *if* $\max(D_1, D_4) < D$.

**Proof :** Let $(s_1(t), x_1(t), s_2(t), x_2(t))$ be a solution of (2). From (3) and (4) we deduce that

$$s_1(t) = s_1^{in} - x_1(t) + K_1 e^{-Dt} \quad \text{and} \quad s_2(t) = s_2^{in} + x_1(t) - x_2(t) + K_2 e^{-Dt},$$

where $K_1 = s_1(0) + x_1(0) - s_1^{in}$ and $K_2 = s_2(0) + x_2(0) - x_1(0) - s_2^{in}$. Hence $(x_1(t), x_2(t))$ is a solution of the nonautonomous system of two differential equations :

$$\begin{cases} \dot{x}_1 = \left[ f_1\left(s_1^{in} - x_1 + K_1 e^{-Dt}, s_2^{in} + x_1 - x_2 + K_2 e^{-Dt}\right) - D \right] x_1, \\ \dot{x}_2 = \left[ f_2\left(s_1^{in} - x_1 + K_1 e^{-Dt}, s_2^{in} + x_1 - x_2 + K_2 e^{-Dt}\right) - D \right] x_2. \end{cases} \tag{9}$$

This is an asymptotically autonomous differential system which converge to the autonomous system (5). The set $\Omega$ is attractor of all trajectories in $\mathbb{R}^4_+$ and the phase portrait of system reduced to $\Omega$ (5) contains only locally stable nodes, unstable nodes, saddle points and no trajectory joining two saddle points. Thus we can apply Thiemes's results [12] and conclude that the asymptotic behaviour of the solution of the complete system (9) is the same that the asymptotic behaviour described for the reduced system (5) and the main result is then deduced. ■

## 3 Numerical simulations

We performed numerical simulations using classical Monod functions to express growth rates as it is often the case in more specific biological models, taking into account the substrate inhibition on the growth of the organisms :

$$f_1(s_1,s_2)=\frac{9s_1}{(1+s_1)(2+s_2)},\qquad f_2(s_1,s_2)=\frac{9s_2}{(2+s_2)(1+s_1)}. \tag{10}$$

For $s_1^{in}=2$ and $s_2^{in}=1$, one can readily check that the functional responses (10) satisfy Assumptions *H1* to *H4* with $D_4=1<D_1=2$. As it is shown in Fig. 4, if $D=0.8$ which satisfies $D<D_4$, the trajectories are

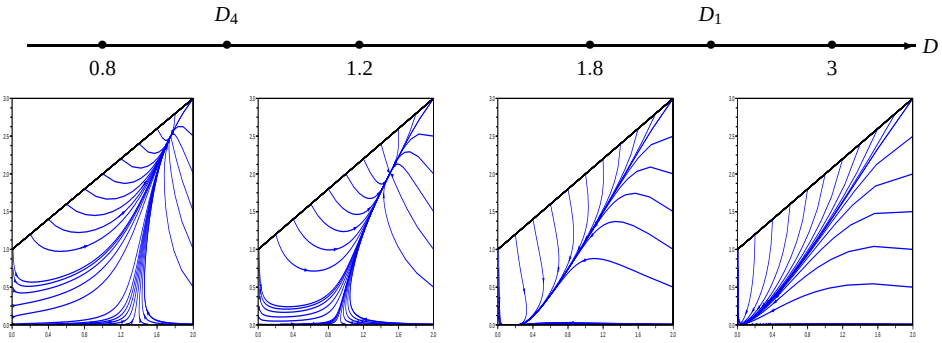


**Fig. 4** $x_1-x_2$ behaviour

converging to the positive equilibrium $F^*=(1.75,2.5)$ and if $D=1.2$ which satisfies $D_4<D<D_1<D_2\simeq 2.56$, the trajectories are converging to the positive equilibrium $F^*=(1.5,2)$ and if $D=1.8$ which satisfies $D_2\simeq 1.692<D<D_1$, the trajectories are converging to the equilibrium $F^1\simeq(0.2087,0)$ and finally if $D=3$ which satisfies $D>D_1$, we have extinction of the two species.

## 4 The anaerobic digestion process : An example of a synthrophic relationship

"Methane fermentation" or "anaerobic digestion" is a process that converts organic matter into a gaseous mixture mainly composed of methane and carbon dioxide ($CH_4$ and $CO_2$) through the action of a complex bacterial ecosystem (cf. Fig.5). It is often used for the treatment of concentrated wastewaters or to stabilize the excess sludge produced in wastewater treatment plants into more stable products. There is also considerable interest in plant-biomass-fed digesters, since the produced methane can be valorized as a source of energy. It is usually considered that a number of metabolic groups of bacteria are involved sequencially.

One specific characteristic of the anaerobic process is that within such groups, there exists populations exhibiting obligatory mutualistic relationships. Such a syntrophic relationship is necessary for the biological reactions to be thermodynamically possible. In the first steps of the reactions (called "acidogenesis"), some hydrogen is produced. In El Hajji et al.[5], this production of hydrogen at this reaction step was neglected (compare Fig.5 with Fig.1 of [5]). This hypothesis constitue the first novelty with respect to [5]. It is to be noticed that an excess of hydrogen in the medium inhibits the growth of another bacterial group called "acetogenic bacteria". Their association with $H_2$ consuming bacteria is thus necessary for the second step of the reaction to be fulfilled. Such a syntrophic relationship has been pointed out in a number of experimental works (cf. for instance the seminal work by [3]). Let us consider the subsystem of the anaerobic system where the VFA (for Volatile Fatty Acids) are transformed into $H_2$, $CH_4$ and $CO2$. We can formalize the corresponding

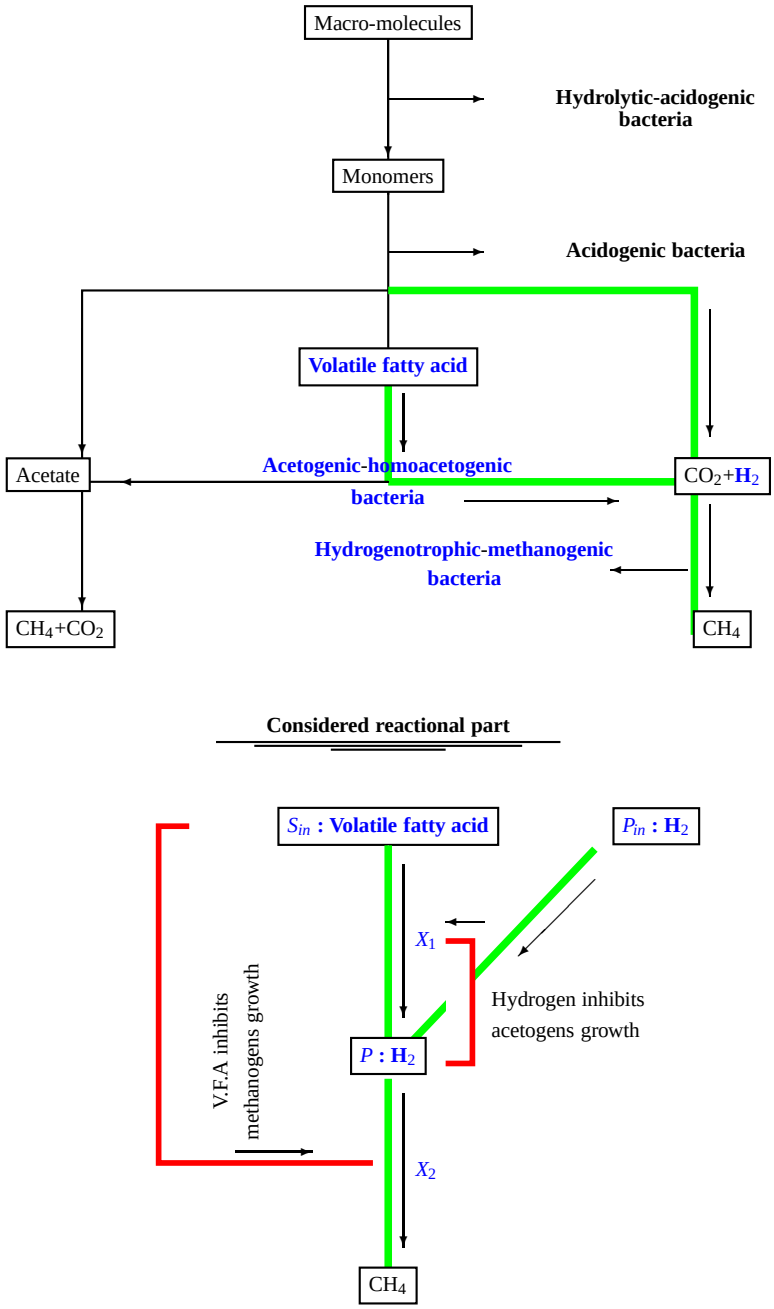


**Fig. 5** Anaerobic fermentation process

biological reactions as a first bacterial consortium $X_1$ (the acetogens) transforming $S_1$ (the VFA) into $S_2$ (the hydrogen) and acetate (cf. Fig.5). Then, a second species $X_2$ (the hydrogenotrophic-methanogenic bacteria) grows on $S_2$. In practice, acetogens are inhibited by an excess of hydrogen and methanogens by an excess of VFA. Thus, it is further assumed that $X_1$ is inhibitied by $S_2$ and $X_2$ by $S_1$. The last inhibition relationship con-

stiute the second novelty with respect to [5]. This situation is precisely the one considered within the model (1).

## 5 Conclusion

We have proposed a mathematical model involving a syntrophic relationship of two bacteria. The analysis of the model is mainly based :

- on Dulac's criterion that rules out the possibility of periodic solutions for the reduced planar system,
- on the Poincaré-Hopf index Theorem to prove the existence and uniqueness of the positive equilibrium point,
- on the application of the Poincaré-Bendixon Theorem,
- on Thieme's results to prove that the stability properties of the reduced planar system are linked with the stability properties of the overall system.

It results from this analysis that, under general and natural assumptions of monotonicity on the functional responses, the stable asymptotic coexistence of the two bacteria is possible.


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