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Abstract

Many microbial ecosystems can be seen as microbial 'food chains' where the different reaction steps can be seen as such: the waste products of the organisms at a given reaction step are consumed by organisms at the next reaction step. In the present paper we study a model of a two-step biological reaction with feedback inhibition, which was recently presented as a reduced and simplified version of the anaerobic digestion model ADM1 of the International Water Association (IWA). It is known that in the absence of maintenance (or decay) the microbial 'food chain' is stable. In a previous study, using a purely numerical approach and ADM1 consensus parameter values, it was shown that the model remains stable when decay terms are added. However, the authors could not prove in full generality that it remains true for other parameter values. In this paper we prove that introducing decay in the model preserves stability whatever its parameters values are and for a wide range of kinetics.

Keywords: Microbial ecosystems, Syntrophic relationship, Maintenance, Stability, Food chains

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1. Introduction

Two-step models are commonly used to describe microbial systems, which take the form of a cascade of two biological reactions where one substrate S_0 is consumed by one microorganism X_0 to produce a product S_1 that serves as the main limiting substrate for a second microorganism X_1 as schematically represented by the following reaction scheme:

$$S_0 \xrightarrow{f_0} X_0 + S_1, \qquad S_1 \xrightarrow{f_1} X_1$$

where f_0 and f_1 are the growth functions that may depend on several substrates. The substrate and biomass concentrations in this two-step model evolve according to the four-dimensional dynamical system of ODEs

$$\left\{\begin{array}{lcl} \dfrac{dS_0}{dt} & = & D\left(S_0^{in} - S_0\right) - f_0\left(\cdot\right) X_0 \\ \dfrac{dX_0}{dt} & = & -\alpha D X_0 + Y_0 f_0\left(\cdot\right) X_0 - a_0 X_0 \\ \dfrac{dS_1}{dt} & = & D\left(S_1^{in} - S_1\right) + Y_2 f_0\left(\cdot\right) X_0 - f_1\left(\cdot\right) X_1 \\ \dfrac{dX_1}{dt} & = & -\alpha D X_1 + Y_1 f_1\left(\cdot\right) X_1 - a_1 X_1 \end{array}\right. \tag{1}$$

where Y_0 are the Yield coefficients. Substrate S_0 and S_1 are introduced with an input concentration S_0^{in} and S_1^{in} respectively, and at dilution rate D. Depending on the technology used to confine the reactions, the coefficient $\alpha \leq 1$ is not necessarily equal to 1 and $1 - \alpha$ represents the proportion of biomass which is retained in the reactor. This model includes the maintenance (or decay) terms a_0 and a_1. Maintenance, in its most general assertion, is the consumption of energy for all processes other than growth: it is modelled either by adding a negative term on the substrate dynamic without associating it to growth or by considering a decay term on the biomass dynamics, as in (1). For more information about the modelling of maintenance, the reader is referred to [16]. These models present the advantage of being complex enough to capture important process properties while being simple enough to be mathematically studied.

When the growth function f_0 depends only on the substrate S_0 and the growth function f_1 depends only on the substrate S_1, that is

$$f_0(\cdot) = f_0(S_0), \qquad f_1(\cdot) = f_1(S_1), \tag{2}$$

the system is known as commensalistic: one species grows on the product of another one [18, 22]. The system has a cascade structure: solve the first and second equations for S_0, X_0, and then use this result is the remaining equations to find S_1, X_1. Consequently S_0 and X_0 are the same in pure and mixed culture experiments. The number of steady-states and their stability as a function of model inputs and parameters may be investigated [4, 5, 21].

When f_0 depends on both substrates S_0 and S_1 and f_1 depends only on S_1, that is

$$f_0(\cdot) = f_0(S_0, S_1), \qquad f_1(\cdot) = f_1(S_1) \tag{3}$$

the system is known as syntrophic. For instance if the first organism is inhibited by high concentrations of the product S_1, the extent to which the substrate S_0 is degraded by the organism X_0 depends on the efficiency of the removal of the product S_1 by the bacteria X_1. The mathematical analysis of such model is more delicate than commensalistic models, see for instance [8, 14, 15, 29] and the more recent papers [10, 11, 19, 26, 30].

A model of a two-tiered microbial 'food chain' with feedback inhibition, which encapsulates the essence of the anaerobic digestion process was recently proposed [30]. Anaerobic digestion is a biological process that converts organic matter into a gaseous mixture composed mainly of methane and carbon dioxide through the action of a complex bacterial and archaeal ecosystem. It is often used for the treatment of concentrated wastewaters or to convert the excess sludge produced in wastewater treatment plants into more stable products [17, 23]. One of its advantages is that the methane produced can be used profitably as a source of energy. It is usually considered that a number of metabolic groups of microorganisms are involved sequentially in several serial and parallel conversion steps to finally produce methane and carbon dioxide. The Anaerobic Digestion Model No. 1 (ADM1) of the IWA Task Group for Mathematical

Modelling of Anaerobic Digestion Processes [3, 12] is too complex to permit mathematical analysis of its nonlinear dynamics and only numerical investigations are available [7].

The model of Xu et al. [30] includes maintenance terms and considers the syntrophic associations between propionate degraders and methanogens. It was shown that the non-trivial steady-state is not necessarily stable. In addition simulation results with the ADM1 consensus values indicate that the positive steady-state is always stable whenever it exists. For the operators of anaerobic wastewater treatment systems the results of Xu et al. [30] show that the syntrophic associations between propionate degraders and methanogens are inherently stable under realistic environmental conditions. However, the possibility of an unstable positive steady-state was not excluded for other parameter values and the title of [30], *Maintenance affects the stability of a two-tiered microbial 'food chain'?* left unanswered the question of the effects of maintenance from a more general viewpoint. In the present paper, we show that for any values of the parameters the positive steady-state is stable as long as it exists, that is to say, maintenance does not affect the stability of the considered two-tiered microbial 'food chain', see [20].

The paper is organized as follows. In Section 2, we review the different two-step models that have been proposed in the literature and we recall what useful informations were obtained for applications from their qualitative mathematical analysis. In Section 3 we present the hypothesis on (1,3). In Section 4 we give the description of the steady-state and their stability. In Section 5 we describe the operating diagram. In Section 6 we apply our results to the model of Xu et al. [30] and we give an answer to open questions on the stability of the positive steady-state of their model. Concluding remarks are given in Section 7. The technical proofs of the results are given in the Appendix.

2. Commensalism, mutualism and syntrophy

The different analyses of the class of models (1) available in the literature essentially differ on the way the growth rate functions are characterized and whether a specific input for S_1 or a coefficient α in the dilution rate of the biomass is considered or not. In most cases, the models used are not generic in the sense either model parameters are fixed or the growth functions are predefined (Monod, Haldane, etc).

Following Stephanopoulos [22] we say that ‘Two populations of microorganisms which grow in a mixed culture and interact in such a way that one population (the commensal population) depends for its growth on the other population and thus benefits from the interaction while the other population (the host) is not affected by the growth of the commensal population constitutes an example of commensalism’. Reilly [18] was the first to propose a mathematical study of a pure commensalistic model (1,2) holds and $a_0 = a_1 = 0$, $\alpha = 1$. He was interested in explaining surprising oscillations observed within the course of an experiment realized in making *Saccharomyces carlsbergensis* growing on fructose produced by *Acetobacter suboxyduns* from mannitol. In particular, he established theoretical conditions involving a feedback from the yeast to the bacteria. In this study, explicit growth functions modelling the proposed feedback were used.

An important contribution on the modelling of anaerobic digestion as a commensalistic system is the model by Bernard et al. [5]. The authors considered a Monod function for f_0 and a Haldane function for f_1. Sbarciog et al. [21] studied this model for $\alpha = 1$ while the interesting case where $0 < \alpha < 1$ and where growth functions were characterized by qualitative properties was studied by Benyahia et al. [4]. Prior to these investigations, and regarding the potential of anaerobic systems to produce renewable energy, the study of these models were particularly important for optimizing anaerobic digestion, notably through the synthesis of state observers and control feedback laws (cf. for instance [1, 2]).

Another fundamental ecological interactions which can be modelled by two-step reaction models with two microorganisms are mutualism and syntrophy. Mutualism is defined as a situation where two organisms cooperate typically in producing mutually the substrate necessary to the growth of the other [9]. A syntrophic relationship between two organisms refers to growth functions of the form (3) where the species exhibit mutualism but where, in contrast to what happens in a purely symbiotic relationship, one of the species can grow without the other. Important results of these studies were conditions under which a stable coexistence may occur. Wilkinson et al. [29] considered the case of growth functions of the form

$$f_0(S_0, S_1) = \frac{m_0 S_0}{K_0 + S_0} \frac{1}{1 + S_1/L_1}, \quad f_1(S_1) = \frac{m_1 S_1}{K_1 + S_1} \tag{4}$$

Kreikenbohm et al. [14] considered the case where f_1 is a Monod function and the growth function f_0 takes the form

$$f_0(S_0, S_1) = \begin{cases} \frac{m_0(S_0 - S1/L)}{K_0 + S_0 + K_1 S_1} & \text{if} \quad S_0 - S1/L > 0 \\ 0 & \text{otherwise} \end{cases} \tag{5}$$

In this case the first organism is unable to grow unless the quotient S_1/S_0 is small enough, say, $S_1 < LS_0$. Burchard [8] extended the results of [14, 29] to a large class of more generic growth functions, including the special cases (4) and (5). He highlighted conditions under which there is persistence or extinction. El Hajji et al. [10], motivated by the analysis of the main studied steps of the anaerobic digestion where H_2-producing acetogens are associated to H_2-utilizing bacteria, considered the general case where the growth functions (3), satisfy the following properties:

$$\frac{\partial f_0}{\partial S_0} > 0, \quad \frac{\partial f_0}{\partial S_1} < 0, \quad \frac{df_1}{dS_1} > 0 \tag{6}$$

Another extension was considered by Kreikenbohm et al. [15], which considered the case where S_0 appears also in $f_1(\cdot)$:

$$f_1(S_0, S_1) = \frac{m_1 S_1}{K_1 + S_1} \frac{1}{1 + S_0/L_0}$$

The mathematical analysis of this model showed the occurrence of bistability that cannot be observed when $f_1(\cdot)$ depends only on S_0. Sari et al. [19] considered the general situation of a growth function $f_1(\cdot) = f_1(S_0, S_1)$, which is increasing in S_1 and decreasing in S_0 and showed, in contrast with the case where $f_1(\cdot) = f_1(S_1)$ depends only on S_1, that a multiplicity of positive equilibria can occur. This work was motivated by the study of the influence of the presence of an input term into the dynamics of S_1 again and by the consideration of more general forms for growth rate functions to investigate the association of H_2-producing acetogens and H_2-utilizing bacteria. Other models for which $f_0(\cdot) = f_0(S_0, S_1)$ and $f_1(\cdot) = f_1(S_0, S_1)$, exhibiting the multiplicity of positive equilibria can be found in [26].

All these studies do not include maintenance terms. This short review of the existing literature shows that under conditions like (6) and without maintenance terms ($a_0 = a_1 = 0$), the positive steady-state is unique and stable, if its exists [8, 10, 14, 29]. On the other hand as soon as $f_1(\cdot) = f_1(S_0, S_1)$ may depend on S_0 then instability of the positive steady-state can occur [15, 19, 26].

To the best of our knowledge, Xu et al. [30] were the first to consider the effects of maintenance terms in (1,3), in the particular case of the growth functions (4), and $S_1^{in} = 0$, $\alpha = 1$. As mentioned in the introduction these authors were not able to show that the positive steady-state is stable if it exists. In the present paper we will consider the general case (1,3) where growth functions satisfy (6) and with maintenance terms ($a_0 > 0$, $a_1 > 0$) and $S_1^{in} = 0$, $\alpha = 1$. We will prove that the positive steady-state is stable whenever it exists. Therefore, in this paper we generalize [30] by allowing a larger class of growth functions, we generalize [29] by allowing a larger class of growth functions and maintenance terms, and we generalize [10] by allowing maintenance terms. For the applications our results show that that the syntrophic associations between propionate degraders and methanogens are inherently stable for a wide range of kinetics and whatever the parameters values are, not only for the kinetics (4) and with the ADM1 consensus values of parameters as shown in [30].

An important and interesting extension should be mentioned here: Weedermann et al. [27] proposed an 8-dimensional mathematical model, which includes syntrophy and inhibition, both mechanisms considered by Bernard et al. [5] and by El Hajji et al. [10]. The effects of maintenance terms are considered by

Weedermann et al. [28].

3. The model

In this paper, we study the model (1,3) with $\alpha = 1$ and $S_1^{in} = 0$. We obtain the following system

$$\left\{\begin{array}{lcl} \dfrac{dS_0}{dt} & = & D\left(S_0^{in} - S_0\right) - f_0\left(S_0, S_1\right) X_0 \\ \dfrac{dX_0}{dt} & = & -DX_0 + Y_0 f_0\left(S_0, S_1\right) X_0 - a_0 X_0 \\ \dfrac{dS_1}{dt} & = & -DS_1 + Y_2 f_0\left(S_0, S_1\right) X_0 - f_1\left(S_1\right) X_1 \\ \dfrac{dX_1}{dt} & = & -DX_1 + Y_1 f_1\left(S_1\right) X_1 - a_1 X_1 \end{array}\right. \tag{7}$$

Notice that we do not assume any specific analytical expression for the growth and inhibition functions. Our analysis will use only the following general assumptions for the growth functions $f_0\left(S_0, S_1\right)$ and $f_1\left(S_1\right)$:

A1 For all $S_0 > 0$ and $S_1 \geq 0$, $f_0\left(S_0, S_1\right) > 0$ and $f_0\left(0, S_1\right) = 0$.

A2 For all $S_1 > 0$, $f_1\left(S_1\right) > 0$ and $f_1(0) = 0$.

A3 For all $S_0 > 0$ and $S_1 > 0$, $\dfrac{\partial f_0}{\partial S_0}\left(S_0, S_1\right) > 0$ and $\dfrac{\partial f_0}{\partial S_1}\left(S_0, S_1\right) < 0$.

A4 For all $S_1 > 0$, $\dfrac{df_1}{dS_1}\left(S_1\right) > 0$.

Hypothesis **A1** signifies that no growth can take place for species X_0 without the substrate S_0. Hypothesis **A1** means that the intermediate product S_1 is necessary for the growth of species X_1. Hypothesis **A3** means that the growth rate of species X_0 increases with the substrate S_0 but it is self-inhibited by the intermediate product S_1. Hypothesis **A4** means that the growth of species X_1 increases with intermediate product S_1 produced by species X_0. Note that this defines a syntrophic relationship between the two species.

To ease the mathematical analysis of the system, we can rescale system (7) using the following change of variables adapted from [19]:

$$s_0 = Y_2 S_0, \quad x_0 = \frac{Y_2}{Y_0} X_0, \quad s_1 = S_1, \quad x_1 = \frac{1}{Y_1} X_1,$$

We obtain the following system

$$\left\{\begin{array}{rcl}\dfrac{ds_0}{dt} & = & D(s_0^{in}-s_0)-\mu_0(s_0,s_1)x_0\\ \dfrac{dx_0}{dt} & = & -Dx_0+\mu_0(s_0,s_1)x_0-a_0x_0\\ \dfrac{ds_1}{dt} & = & -Ds_1+\mu_0(s_0,s_1)x_0-\mu_1(s_1)x_1\\ \dfrac{dx_1}{dt} & = & -Dx_1+\mu_1(s_1)x_1-a_1x_1\end{array}\right. \tag{8}$$

where $s_0^{in}=Y_2S_0^{in}$ and μ_0 and μ_1 are defined by

$$\mu_0(s_0,s_1)=Y_0f_0\left(\frac{1}{Y_2}s_0,s_1\right) \quad \text{and} \quad \mu_1(s_2)=Y_1f_1(s_1) \tag{9}$$

The functions μ_0 and μ_1 are general functions with their own properties. Since the functions f_0 and f_1 satisfy hypotheses **A1**–**A4**, it follows from (9) that functions μ_0 and μ_1 satisfy:

H1 For all $s_0>0$ and $s_1\geq 0$, $\mu_0(s_0,s_1)>0$ and $\mu_0(0,s_1)=0$.

H2 For all $s_1>0$, $\mu_1(S_1)>0$ and $\mu_1(0)=0$.

H3 For all $s_0>0$ and $s_1>0$, $\dfrac{\partial\mu_0}{\partial s_0}(s_0,s_1)>0$ and $\dfrac{\partial\mu_0}{\partial s_1}(s_0,s_1)<0$.

H4 For all $s_1>0$, $\dfrac{d\mu_1}{ds_1}(s_1)>0$.

It should be noticed that (8) was studied in [10, 19] in the case where maintenance effects are not taken into account, i.e. $a_0=a_1=0$. We can easily prove that that for every non-negative initial condition, the solution of (8) has non-negative components and is positively bounded and thus is defined for every positive t.

4. Steady-state and stability analysis

A steady-state of (8) is a solution of the following nonlinear algebraic system obtained from (8) by setting the right-hand sides equal to zero:

$$D(s_0^{in}-s_0)-\mu_0(s_0,s_1)x_0 \quad = \quad 0 \tag{10}$$

$$-Dx_0+\mu_0(s_0,s_1)x_0-a_0x_0 \quad = \quad 0 \tag{11}$$

$$-Ds_1 + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1 = 0 \tag{12}$$

$$-Dx_1 + \mu_1(s_1)x_1 - a_1x_1 = 0 \tag{13}$$

A steady-state exists (or is said to be 'meaningful' [30]) if and only if all its components are non-negative. From equation (11) we deduce that:

$$x_0 = 0 \quad \text{or} \quad \mu_0(s_0, s_1) = D + a_0 \tag{14}$$

and from equation (13) we deduce that:

$$x_1 = 0 \quad \text{or} \quad \mu_1(s_1) = D + a_1 \tag{15}$$

The case $x_0 = 0$ and $x_1 > 0$ is excluded. Indeed, as a consequence of (15), we have $\mu_1(s_1) = D + a_1$ and, as a consequence of (12), we have $Ds_1 + (D+a_1)x_1 = 0$, which is impossible since $s_1 \geq 0$ and $x_1 > 0$. Therefore, three cases must be distinguished:

SS0: $x_0 = 0$, $x_1 = 0$ where both species are washed out.

SS1: $x_0 > 0$, $x_1 = 0$, where species x_1 is washed out while x_0 survives.

SS2: $x_0 > 0$, $x_1 > 0$, where both species survive.

For the description of the steady-states and their stability, we need the following notations. Since the function $s_1 \mapsto \mu_1(s_1)$ is increasing, it has an inverse function $y \mapsto M_1(y)$, so that, for all $s_1 \geq 0$ and $y \in [0, \sup \mu_1(\cdot))$

$$s_1 = M_1(y) \Longleftrightarrow y = \mu_1(s_1) \tag{16}$$

Let s_1 be fixed. Since the function $s_0 \mapsto \mu_0(s_0, s_1)$ is increasing, it has an inverse function $y \mapsto M_0(y, s_1)$, so that, for all $s_0, s_1 \geq 0$, and $y \in [0, \sup \mu_0(\cdot, s_1))$

$$s_0 = M_0(y, s_1) \Longleftrightarrow y = \mu_0(s_0, s_1) \tag{17}$$

The inverse functions $s_1 = M_1(y)$ and $s_0 = M_0(y, s_1)$ can be calculated explicitly in the case of the Monod growth functions (22) considered in Section 6, see formulas (24,(25)). We define the functions:

$$\begin{aligned} F_0(D) &= M_0(D + a_0, 0) \\ F_1(D) &= M_1(D + a_1) + M_0\left(D + a_0, M_1(D + a_1)\right) \end{aligned} \tag{18}$$

Steady-state	Existence condition	Stability condition
SS0	Always exists	$s_0^{in} < F_0(D)$
SS1	$s_0^{in} > F_0(D)$	$s_0^{in} < F_1(D)$
SS2	$s_0^{in} > F_1(D)$	Always Stable

Table 1: Existence and local stability of steady-states.

Notice that $F_1(D) > F_0(D)$ for all $D \geq 0$, as long as they are both defined with the exception $F_1(0) = F_0(0)$, which holds if and only if $a_0 = a_1 = 0$. Now, we can describe the steady-states of (8).

Proposition 1. *Assume that assumptions* **H1**–**H4** *hold. Then (8) has at most three steady-states:*

- *SS0*$=\left(s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0\right)$
  It always exists. It is stable if and only if $s_0^{in} < F_0(D)$.
- *SS1*$=\left(s_0, x_0 = \frac{D}{D+a_0}\left(s_0^{in} - s_0\right), s_1 = s_0^{in} - s_0, x_1 = 0\right)$
  where s_0 *is the solution of equation* $\mu_0(s_0, s_0^{in} - s_0) = D + a$. *It exists if and only if* $s_0^{in} > F_0(D)$. *It is stable if and only if* $s_0^{in} < F_1(D)$.
- *SS2*$=\left(s_0, x_0 = \frac{D}{D+a_0}\left(s_0^{in} - s_0\right), s_1, x_1 = \frac{D}{D+a_1}\left(s_0^{in} - s_0 - s_1\right)\right)$
  where $s_1 = M_1(D + a_1)$ *and* $s_0 = M_0\left(D + a_0, M_1(D + a_1)\right)$. *It exists if and only if* $s_0^{in} > F_1(D)$. *It is stable if it exists.*

The proof is given in the Appendix.

Notice that SS1 exists as soon as SS0 becomes unstable and SS2 exists as soon as SS1 becomes unstable. One concludes that for any value of the operating parameters, there is always one, and only one, steady-state which is stable. The results are summarized in Table 1. When decay effects are not taken into account, i.e. $a_0 = a_1 = 0$, the system can be reduced to a planar system and global stability results can be obtained [10, 19]: for any pair of operating parameters, there is always one, and only one, steady-state which is globally asymptotically stable.

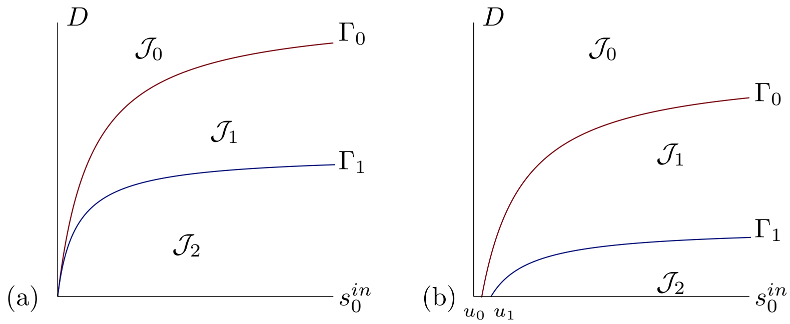

Figure 1: Operating diagram without (a) and with (b) maintenance effects. The values u_0 and u_1 are defined by (19)

5. Operating diagram

The operating diagram shows how the system behaves when we vary the two control parameters S_0^{in} and D. Let $F_0(D)$ and $F_1(D)$ be the functions defined by (18). The curve Γ_0 of equation $s_0^{in} = F_0(D)$ is the border which makes SS0 unstable and at the same time SS1 exists (the red curve on Fig. 1). The curve Γ_1 of equation $s_0^{in} = F_1(D)$ is the border which makes SS1 unstable and at the same time SS2 exists (the blue curve on Fig. 1).

The curves Γ_0 and Γ_1 separate the operating plane (s_0^{in}, D) in three regions, as shown in Fig. 1, labelled $\mathcal{J}_0$, $\mathcal{J}_1$ and $\mathcal{J}_2$. The results of Prop. 1 are summarized in Table 2 which shows the existence and stability of the steady-states SS0, SS1 and SS2 in the regions $\mathcal{J}_0$, $\mathcal{J}_1$ and $\mathcal{J}_2$ of the operating diagram.

The values u_0 and u_1 plotted on the figure are obtained as follows:

$$u_0 = F_0(0) = M_0(a_0, 0), \; u_1 = F_1(0) = \beta + M_0(a_0, \beta), \; \beta = M_1(a_1) \quad (19)$$

If $a_0 \geq \sup_{s_0>0} \mu_0(s_0, 0)$, $F_0(0)$ is not defined and we let $u_0 = +\infty$. In this case the regions $\mathcal{J}_1$ and $\mathcal{J}_2$ are empty. If $a_1 < \sup_{s_1>0} \mu_1(s_1)$ or $a_0 \geq \sup_{s_0>0} \mu_0(s_0 - \beta, \beta)$, $F_1(0)$ is not defined and we let $u_1 = +\infty$. In this case the region $\mathcal{J}_2$ is empty. When maintenance effects are not taken into consideration, then $u_0 = u_1 = 0$ and we have

$$F_0(D) = M_0(D, 0), \quad F_1(D) = M_1(D) + M_0(D, M_1(D))$$

Condition	Region	SS0	SS1	SS2
$s_0^{in} < F_0(D)$	$(s_0^{in}, D) \in \mathcal{J}_0$	S		
$F_0(D) < s_0^{in} < F_1(D)$	$(s_0^{in}, D) \in \mathcal{J}_1$	U	S	
$F_1(D) < s_0^{in}$	$(s_0^{in}, D) \in \mathcal{J}_2$	U	U	S

Table 2: Existence and local stability of steady-states. The letter S (resp. U) means stable (resp. unstable). No letter means that the steady-state does not exist.

6. A two-tiered microbial 'food chain'

The model considered in [30] involves a two-tiered microbial 'food chain' with feedback inhibition, consisting of a propionate degrader and a hydrogenotrophic methanogen. The propionate degrader produces hydrogen which inhibits its own growth. Using the notations of ADM1 the model can be written as

$$\left\{\begin{array}{rcl} \dfrac{dS_{pro}}{dt} &=& D\left(S_{pro,in} - S_{pro}\right) - f_0\left(S_{pro}, S_{H_2}\right) X_{pro} \\ \dfrac{dX_{pro}}{dt} &=& -DX_{pro} + Y_{pro} f_0\left(S_{pro}, S_{H_2}\right) X_{pro} - k_{dec,pro} X_{pro} \\ \dfrac{dS_{H_2}}{dt} &=& -DS_{H_2} + 0.43\left(1 - Y_{pro}\right) f_0\left(S_{pro}, S_{H_2}\right) X_{pro} - f_1\left(S_{H_2}\right) X_{H_2} \\ \dfrac{dX_{H_2}}{dt} &=& -DX_{H_2} + Y_{H_2} f_1\left(S_{H_2}\right) X_{H_2} - k_{dec,H_2} X_{H_2} \end{array}\right. \tag{20}$$

where S_{pro} and X_{pro} are propionate substrate and biomass concentrations; S_{H_2} and X_{H_2} are those for hydrogen; Y_{pro} and Y_{H_2} are the Yield coefficients and $0.43\left(1 - Y_{pro}\right)$ represents the part which goes to hydrogen substrate. Both growth functions take Monod form with an hydrogen inhibition for the first one

$$f_0\left(S_{pro}, S_{H_2}\right) = \frac{k_{m,pro} S_{pro}}{K_{s,pro} + S_{pro}} \frac{1}{1 + \frac{S_{H_2}}{K_{I,H_2}}}, \quad f_1\left(S_{H_2}\right) = \frac{k_{m,H_2} S_{H_2}}{K_{s,H_2} + S_{H_2}} \tag{21}$$

Here, apart from the two operating (or control) parameters, which are the inflowing propionate concentration $S_{pro,in}$ and the dilution rate D, that can vary, all others have biological meaning and are fixed depending on the organisms and substrate considered [see 30, Table 1]. The aim of Xu et al. [30] was to study the stability of the steady-states of the model (20,21) while varying the two operating (or control) parameters D and $S_{pro,in}$. The system (20,21) can have at most three steady-states: a trivial solution where both populations are washed out (SS0), a solution where X_{H_2} is washed out while X_{pro} survives (SS1) and a positive solution where both populations survive (SS2). The local stability of each steady-state was tested by linearisation around the steady-state values of the variables.

The basic results of the analysis of [30] are: for any pair of values of operating parameters, at most one steady-state is stable. When one of the decay terms is not taken into account, i.e. $k_{dec,pro} = 0$ or $k_{dec,H_2} = 0$ in (20), there is always one and only one steady-state which is stable and SS2 is stable as long as it exists. When both decay effects are present, i.e. $k_{dec,pro} > 0$ and $k_{dec,H_2} > 0$ in (20), the authors were not able to check all the Routh-Hurwitz criteria for SS2. They claimed that SS2 is not necessarily stable in theory when it exists and they established numerically that with the ADM1 parameters values, SS2 is stable as long as it exists. However they did not give any values for the biological parameters for which, under some operating parameters, SS2 becomes unstable. As a consequence of Proposition 1, we can say that, for all values of the parameters, SS2 is stable whenever it exits, which actually gives an answer to the questions asked by [30] in their paper.

More precisely, using the following simplified notations in (20)

$$S_0 = S_{pro}, \quad S_0^{in} = S_{pro,in}, \quad S_1 = S_{H_2}, \quad X_0 = X_{pro}, \quad X_1 = X_{H_2}$$

$$Y_0 = Y_{pro}, \quad Y_1 = Y_{H_2}, \quad Y_2 = 0.43\,(1 - Y_{pro}), \quad a_0 = k_{dec,pro}, \quad a_1 = k_{dec,H_2}$$

and using the rescaling (9) and the biological parameters in (21) we obtain the model (8) with the following growth function:

$$\mu_0\,(s_0, s_1) = \frac{m_0 s_0}{K_0 + s_0}\frac{1}{1 + s_1/K_i}, \quad \mu_1\,(s_1) = \frac{m_1 s_1}{K_1 + s_1} \tag{22}$$

where

$$\begin{aligned} &m_0 = Y_0 k_{m,pro}, \quad K_0 = Y_2 K_{s,pro}, \quad K_i = K_{I,H_2} \\ &m_1 = Y_1 k_{m,H_2}, \quad K_1 = K_{s,H_2} \end{aligned} \tag{23}$$

Let us describe our results in the particular case (8,22). Notice that the growth functions (22) satisfy Assumptions **H1**–**H4**, so that Proposition 1 holds. In this case the inverse functions $M_1(y)$ and $y \mapsto M_0(y, s_1)$ of the functions $\mu_1(s_1)$ and $s_0 \mapsto \mu_0(s_0, s_1)$ can be calculated explicitly: we have

$$y \in [0, m_1) \mapsto M_1(y) = \frac{K_1 y}{m_1 - y}, \tag{24}$$

$$y \in \left[0, \frac{m_1}{1 + s_1/K_i}\right) \mapsto M_0(y, s_1) = \frac{K_0 y}{\frac{m_0}{1+s_1/K_i} - y} \tag{25}$$

Therefore, the functions $F_1(D)$ and $F_2(D)$ defined by (18) are given explicitly by

$$\begin{aligned} F_0(D) &= \frac{K_0(D + a_0)}{m_1 - D - a_0} \\ F_1(D) &= \frac{K_1(D + a_1)}{m_1 - D - a_1} + \frac{K_0(D + a_0)}{\frac{m_0}{1 + \frac{K_1(D+a_1)}{(m_1-D-a_1)K_i}} - D - a_0} \end{aligned} \tag{26}$$

Notice that F_0 is defined on $[0, m_1 - a_0)$ and F_1 is defined on $[0, D^+)$ with $D^+ < m_1 - a_0$. On the other hand, the solution s_0 of equation $\mu_0(s_0, s_0^{in} - s_0) = D + a_0$, which is used in SS1, is simply the positive solution of the quadratic equation:

$$m_0 s_0 = (D + a_0)(K_0 + s_0)\left(1 + \frac{s_0^{in} - s_0}{K_i}\right) \tag{27}$$

As a corollary of Proposition 1 we have the following result.

Proposition 2. *Assume that μ_0 and μ_1 are given by (22). Let $F_0(D)$ and $F_1(D)$ be defined by (26). Then (8) has at most three steady-states*

- *SS0*$=\left(s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0\right)$
  It always exists. It is stable if and only if $s_0^{in} < F_0(D)$.
- *SS1*$=\left(s_0, x_0 = \frac{D}{D+a_0}\left(s_0^{in} - s_0\right), s_1 = s_0^{in} - s_0, x_1 = 0\right)$
  where s_0 is the positive solution of the quadratic equation (27). It exists if and only if $s_0^{in} > F_0(D)$. If it exists then it is stable if and only if $s_0^{in} < F_1(D)$.
- *SS2*$=\left(s_0, x_0 = \frac{D}{D+a_0}\left(s_0^{in} - s_0\right), s_1, x_1 = \frac{D}{D+a_1}\left(s_0^{in} - s_0 - s_1\right)\right)$
  where
  $$s_1 = \frac{K_1(D + a_1)}{m_1 - D - a_1}, \qquad s_0 = \frac{K_0(D + a_0)}{\frac{m_0}{1 + \frac{s_1}{K_i}} - D - a_0}$$

It exists if and only if $s_0^{in} > F_1(D)$. It is stable if it exists.

As a consequence of this result we obtain the results of [30]. To make the comparison possible the reader is advised on the main difference between our approach and [30]: we use the rescaling (9) and hence work with the growth functions (22), while Xu et al. [30] use a dimensionless rescaling. Despite this difference, both approaches are equivalent and hence must give the same results. Our quadratic equation (27) used in the description of SS1 is the same as their quadratic equation (A.1), or the quadratic equation without numbering preceding equation (B.1) [see 30, Appendix A and B].

Parameters	Units	Nominal Value
m_0	d^{-1}	0.52
K_0	$\mathrm{kg\,COD/m^3}$	0.124
m_1	d^{-1}	2.10
K_1	$\mathrm{kg\,COD/m^3}$	$2.5\ 10^{-5}$
K_i	$\mathrm{kg\,COD/m^3}$	$3.5\ 10^{-6}$
a_0	d^{-1}	0.02
a_1	d^{-1}	0.02

Table 3: Nominal parameters values.

For the numerical simulations we will use the nominal values of Table 3 obtained from Table 1 of [30] by using the formulas (23) and $a_0 = k_{dec,pro}$, $a_1 = k_{dec,H_2}$. For these values of the parameters, the values u_0 and u_1 are very small, see Fig. 2. Notice that the scaling on the two coordinates in Fig. 2 are different from those of Fig. 2 of [30], since these authors used another rescaling.

7. Discussion

Following [30], we considered a two-tiered 'food chain' with feedback inhibition, which is a generalized model describing the syntrophic interaction of a propionate degrader and a hydrogenotropic methanogen. In the absence of

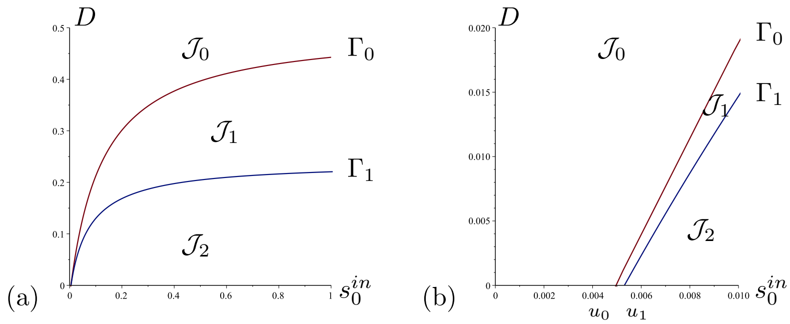

Figure 2: Operating diagram of the model (8)-(22). (a) The model was parametrised with the ADM1 consensus values listed in Table 3. (b) A magnification showing the values $u_0 = 49.6\,10^{-4}$, $u_1 = 53.1\,10^{-4}$ defined by (19).

maintenance these authors proved that this two-tiered 'food chain' is always stable. When maintenance is included in the model they were not able to check the Routh-Hurwitz criteria, and since the possibility of having at least one pair of complex eigenvalues with positive real parts is not theoretically excluded, they concluded that Hopf bifurcation can originate from SS2 [see 30, Appendix B]. However, using the consensus parameters of ADM1 and numerical simulations, they have shown that the model of the methanogenic two-tiered propionate-hydrogen food chain is always stable [see 30, Section 6.2]. In this work we have generalized the model of the two-tiered 'food chain' of [30] by considering generic growth functions and we established the stability of the generalized model with maintenance terms.

In [30], the authors point out that introducing decay or maintenance in the classical predator-prey models results in instability and chaos [13]. For more details on food-chains in the chemostat the reader may consult [6, 24, 25]. Therefore, they observed that, in spite of the fundamental differences between their 'food chain' and the classical predator-prey models, the same intrinsic effect of maintenance on the stability of the food chain is observed [see 30, Section 7]: When maintenance is included in its description, the two-tiered generalized 'food chain' is not necessarily stable in theory. The results obtained in the present paper indicate that the two-tiered generalized 'food chain' is always stable, so that it is fundamentally different from the classical predator-prey model.

It should be noticed that the rescaling used by [30] gives a dimensionless model. However, our present rescaling (9) does not yield a dimensionless model. The new variables s_0, x_0, s_1 and x_1 have the same dimensions as the original variables S_0, X_0, S_1 and X_1. The original growth functions (21) are transformed by our rescaling (9) in the growth functions (22) with the same scale imbalance in the half-saturation rates, see Table 3. We cannot benefit from the dimensionless rescaling used by Xu et al. [30], because this rescaling uses some kinetics parameters of the specific growth functions (21) while we work with general *unspecified* growth functions. The benefit of our rescaling (9) is that it permits to fix the constant yields parameters Y_0, Y_1 and Y_2 in (7) to 1, as shown by the rescaled model (8).

We were successful in checking the Routh-Hurwitz criteria because we work with general growth functions (defined by their qualitative properties given in assumptions **A1**–**A4**) and our computations are not encumbered by the specific form of the growth functions considered by Xu et al. [30]. These authors noticed [see 30, Section 7] that direct application of symbolic analysis programs, such as Maple or Mathematica, did not provide adequate solutions for the stability of the system. Actually we used the symbolic analysis program Maple to verify that the coefficients β_i in the expression of the term $f_1f_2f_3 - f_1^2f_4 - f_3^2$ given in Appendix D are correct. It should be noticed that [30] have claimed [see 30, Remark 1] that their method is still effective for other growth functions. Our main contribution was to believe them and to try to solve the problem with general growth functions.

In the model (7) considered in this work, the first species X_0 uses the substrate S_0 for its growth and produces a substrate S_1 consumed by the second species X_1 for its growth. The substrate S_1 produced by the first species inhibits its own growth, that is, the growth function $f_0(S_0, S_1)$ is decreasing with respect to S_1. In practice, and in many complex models as the ADM1, it happens that the second species is also inhibited by the first substrate. Thus, it is interesting to consider the case where the second species is inhibited by the substrate S_0, namely that $f_1(S_0, S_1)$ also depends on S_0 and is decreasing with respect to S_0. It has been shown by Sari et al. [19] that the introduction of this last inhibiting relationship in the model completely changes the model properties while maintenance was not considered. In particular, the modified model exhibits multiplicity of positive steady-states. However, it should be stressed that these results were very general: whether this instability occurs for realistic environmental conditions or not is under investigation.

Another interesting question, which is the object of a future work, is to consider an input term S_1^{in} in (7), as well as a coefficient $\alpha < 1$ in the dilution rate of the biomass, as it was the case in the general setting of (1). For instance if $S_1^{in} > 0$ then there exists an additional steady-state where $X_0 = 0$ is washed out and $X_1 > 0$ does not go to extinction.

Appendix A. Stability analysis

We give the proof of Prop. 1. A steady-state (s_0, x_0, s_1, x_1) of (8) is a solution of the set of algebraic equations (10-13). The local stability of each steady-state depends on the sign of the real parts of the eigenvalues of the corresponding Jacobian matrix for the system (8). This is the matrix of the partial derivatives of the right hand side with respect to the state variables evaluated at the given steady-state (s_0, x_0, s_1, x_1), that is:

$$J = \begin{bmatrix} -D - Ex_0 & -\mu_0 & Fx_0 & 0 \\ Ex_0 & \mu_0 - D - a_0 & -Fx_0 & 0 \\ Ex_0 & \mu_0 & -D - Fx_0 - Gx_1 & -\mu_1 \\ 0 & 0 & Gx_1 & \mu_1 - D - a_1 \end{bmatrix} \tag{A.1}$$

where

$$E = \frac{\partial \mu_0}{\partial s_0}(s_0, s_1) > 0, \quad F = -\frac{\partial \mu_0}{\partial s_1}(s_0, s_1) > 0, \quad G = \frac{d\mu_1}{ds_1}(s_1) > 0$$

The eigenvalues of J are the roots of its characteristic polynomial $\det(J - \lambda I)$. Notice that we have used the opposite sign for the partial derivative $F =$

$-\frac{\partial \mu_0}{\partial s_1}(s_0, s_1)$, so that all constants involved in the computations become positive, which will simplify the analysis of the characteristic polynomial of J.

Appendix B. SS0

At SS0, $x_0 = 0$, $x_1 = 0$. As a result of (10) and (12), $s_0 = s_0^{in}$ and $s_1 = 0$. SS0 always exists. Evaluated at SS0, the Jacobian matrix (A.1) becomes

$$J = \begin{bmatrix} -D & -\mu_0(s_0^{in}, 0) & 0 & 0 \\ 0 & \mu_0(s_0^{in}, 0) - D - a_0 & 0 & 0 \\ 0 & \mu_0(s_0^{in}, 0) & -D & 0 \\ 0 & 0 & 0 & -D - a_1 \end{bmatrix}$$

Its eigenvalues are $\lambda_1 = \mu_0(s_0^{in}, 0) - D - a_0$, $\lambda_2 = -D - a_1$ and $\lambda_3 = \lambda_4 = -D$. For being stable we need $\lambda_1 < 0$. Therefore SS0 is unstable if and only if

$$\mu_0(s_0^{in}, 0) > D + a_0 \tag{B.1}$$

Since the function $s_0 \mapsto \mu_0(s_0, 0)$ is increasing, and using (17) we have the following equivalence

$$\mu_0(s_0^{in}, 0) > D + a_0 \Longleftrightarrow s_0^{in} > M_0(D + a_0, 0)$$

Therefore, according to (18), (B.1) is equivalent to $s_0^{in} > F_0(D)$.

Appendix C. SS1

At SS1, $x_0 \neq 0$, $x_1 = 0$. As a consequence of (14) $\mu_0(s_0, s_1) = D + a_0$. As a result of (10) and (12)

$$D(s_0^{in} - s_0) = \mu_0(s_0, s_1)x_0 \quad \text{and} \quad Ds_1 = \mu_0(s_0, s_1)x_0$$

Hence $x_0 = \frac{D}{D+a_0}\left(s_0^{in} - s_0\right)$ and $D(s_0^{in} - s_0) = Ds_1$, so that $s_0 + s_1 = s_0^{in}$. Therefore s_0 is a solution of equation

$$\mu_0(s_0, s_0^{in} - s_0) = D + a_0 \tag{C.1}$$

SS1 exists if and only if this equation has a solution in the interval $(0, s_0^{in})$. The function $s_0 \mapsto \psi(s_0) = \mu_0(s_0, s_0^{in} - s_0)$ is increasing since its derivative $\frac{d\psi}{ds_0} = \frac{\partial \mu_0}{\partial s_0} - \frac{\partial \mu_0}{\partial s_1} >$ is positive. Using $\psi(0) = 0$ and $\psi(s_0^{in}) = \mu(s_0^{in}, 0)$ we conclude that equation (C.1) has a solution in the interval $(0, s_0^{in})$ if and only if $\psi(s_0^{in}) = \mu(s_0^{in}, 0) > D + a_0$, that is to say condition (B.1) holds. The condition of existence of SS1 is then equivalent to the condition of instability of SS0. Evaluated at SS1, the Jacobian matrix (A.1) becomes:

$$J = \begin{bmatrix} -D - Ex_0 & -D - a_0 & Fx_0 & 0 \\ Ex_0 & 0 & -Fx_0 & 0 \\ Ex_0 & D + a_0 & -D - Fx_0 & -\mu_1 \\ 0 & 0 & 0 & \mu_1 - D - a_1 \end{bmatrix}$$

Its characteristic polynomial is:

$$\det(J - \lambda I) = (\lambda - \mu_1 + D + a_1)(\lambda + D)\left(\lambda^2 + [D + (E + F)x_0]\,\lambda + (D + a_0)(E + F)x_0\right)$$

Its eigenvalues are $\lambda_1 = \mu_1 - D - a_1$, $\lambda_2 = -D$ and λ_3 and λ_4 are the roots of the following quadratic equation:

$$\lambda^2 + [D + (E + F)x_0]\,\lambda + (D + a_0)(E + F)x_0 = 0$$

Since $\lambda_3\lambda_4 = (D + a_0)(E + F)x_0 > 0$ and $\lambda_3 + \lambda_4 = -[D + (E + F)x_0] < 0$, the real parts of λ_3 and λ_4 are negative. So for being stable it must be $\lambda_1 < 0$. Therefore SS1 is stable if and only if

$$\mu_1(s_0^{in} - s_0) < D + a_1, \text{ where } s_0 \text{ is the solution of (C.1)} \tag{C.2}$$

Since the function $s_1 \mapsto \mu_1(s_1)$ is increasing, we have the following equivalence

$$\mu_1(s_0^{in} - s_0) < D + a_1 \Longleftrightarrow s_0 < s_0^{in} - M_1(D + a_1)$$

Since the function $s_0 \mapsto \psi(s_0) = \mu_0\left(s_0, s_0^{in} - s_0\right)$ is decreasing, we deduce that $\psi(s_0) > \psi\left(s_0^{in} - M_1(D + a_1)\right)$. Since s_0 be the solution of (C.1),

$$\psi(s_0) = \mu_0\left(s_0, s_0^{in} - s_0\right) = D + a_0$$

Therefore, the condition (C.2) of stability of SS1 is equivalent to:

$$D + a_0 < \mu_0 \left(s_0^{in} - M_1(D + a_1), M_1(D + a_1)\right) \tag{C.3}$$

Since the function $s_0 \mapsto \mu_0 (s_0, M_1(D + a_1))$ is increasing, and using (17), the condition (C.3) is equivalent to

$$s_0^{in} - M_1(D + a_1) < M_0 (D + a_0, M_1(D + a_1))$$

which is, according to (18), equivalent to

$$s_0^{in} < M_1(D + a_1) + M_0 (D + a_0, M_1(D + a_1)) =: F_1(D)$$

Appendix D. SS2

At SS2, $x_0 \neq 0$, $x_1 \neq 0$. As a consequence of (14) and (15) s_0 and s_1 are solutions of the set of equations

$$\mu_0(s_0, s_1) = D + a_0, \qquad \mu_1(s_1) = D + a_1$$

Using (16) we obtain $s_1 = M_1(D + a_1)$ and s_0 is a solution of equation

$$\mu_0 (s_0, M_1(D + a_1)) = D + a_0 \tag{D.1}$$

Using (17) we obtain $s_0 = M_0 (D + a_0, M_1(D + a_1))$. As a result of (10) and (12)

$$x_0 = \frac{D}{D + a_0} \left(s_0^{in} - s_0\right), \qquad x_1 = \frac{D}{D + a_1} \left(s_0^{in} - s_0 - s_1\right)$$

SS2 exists if and only if $s_0^{in} > s_0 + s_1$, that is

$$s_0^{in} > M_1(D + a_1) + M_0 (D + a_0, M_1(D + a_1)) =: F_1(D)$$

Evaluated at SS2, the Jacobian matrix (A.1) becomes:

$$J = \begin{bmatrix} -D - Ex_0 & -D - a_0 & Fx_0 & 0 \\ Ex_0 & 0 & -Fx_0 & 0 \\ Ex_0 & D + a_0 & -D - Fx_0 - Gx_1 & -D - a_1 \\ 0 & 0 & Gx_1 & 0 \end{bmatrix}$$

Its characteristic polynomial is:

$$\det(J - \lambda I) = \lambda^4 + f_1\lambda^3 + f_2\lambda^2 + f_3\lambda + f_4$$

where

$$f_1 = Gx_1 + (E+F)x_0 + 2D$$

$$f_2 = EGx_0x_1 + (2D + a_0)(E+F)x_0 + (2D + a_1)Gx_1 + D^2$$

$$f_3 = (2D + a_0 + a_1)EGx_0x_1 + D(D + a_0)(E+F)x_0 + D(D + a_1)Gx_1$$

$$f_4 = (D + a_0)(D + a_1)EGx_0x_1$$

Hence

$$f_i > 0 \text{ for } i = 1 \cdots 4 \tag{D.2}$$

Since the quantity $E+F$ occurs so often in the computations, we use the notation $H = E + F$. Straightforward calculations show that:

$$f_1f_2 - f_3 = 2D^3 + \alpha_2 D^2 + \alpha_1 D + \alpha_0$$

where

$$\alpha_2 = 4(Hx_0 + Gx_1)$$

$$\alpha_1 = 2(Hx_0 + Gx_1)^2 + a_0Hx_0 + a_1Gx_1$$

$$\alpha_0 = EG(Hx_0 + Gx_1)x_0x_1 + a_0H^2x_0^2 + (a_0 + a_1)FGx_0x_1 + a_1G^2x_1^2$$

Thus

$$f_1f_2 - f_3 > 0 \tag{D.3}$$

On the other hand we have

$$f_1f_2f_3 - f_1^2f_4 - f_3^2 = \beta_5D^5 + \beta_4D^4 + \beta_3D^3 + \beta_2D^2 + \beta_1D + \beta_0$$

where

$$\beta_5 = 2(Hx_0 + Gx_1)$$

$$\beta_4 = 4(Hx_0 + Gx_1)^2 + 2a_0Hx_0 + 2a_1Gx_1$$

$$
\begin{aligned}
\beta_3 &= 2(Hx_0 + Gx_1)^3 + 4EG(Hx_0 + Gx_1)x_0x_1 \\
&\quad + 5a_0H^2x_0^2 + (a_0 + a_1)(3E + 5F)Gx_0x_1 + 5a_1G^2x_1^2
\end{aligned}
$$

$$
\begin{aligned}
\beta_2 &= 4EG(Hx_0 + Gx_1)^2x_0x_1 \\
&\quad + 3a_0H^3x_0^3 + (a_0E + 2a_1E + 6a_0H + 3a_1F)GHx_0^2x_1 \\
&\quad + (2a_0E + a_1E + 3a_0F + 6a_1H)G^2x_0x_1^2 + 3a_1G^3x_1^3 \\
&\quad + a_0^2F(F + 2E)x_0^2 + (a_0Ex_0 - a_1Gx_1)^2 + 2a_0a_1GFx_0x_1
\end{aligned}
$$

$$
\begin{aligned}
\beta_1 &= 2E^2G^2(Hx_0 + Gx_1)x_0^2x_1^2 + (4a_0 + a_1)EGH^2x_0^3x_1 \\
&\quad + (a_0 + a_1)(3E + 5F)EG^2x_0^2x_1^2 + (a_0 + 4a_1)EG^3x_0x_1^3 \\
&\quad + a_0^2(3E^2 + 3EF + F^2)Fx_0^3 + a_0(2a_0E + a_0F + 2a_1F)GFx_0^2x_1 \\
&\quad + (Ex_0 + Gx_1)(a_0Ex_0 - a_1Gx_1)^2 + (2a_0a_1 + a_1^2)G^2Fx_0x_1^2
\end{aligned}
$$

$$
\begin{aligned}
\beta_0 &= (a_0 + a_1)E^2G^2(Hx_0 + Gx_1)x_0^2x_1^2 + a_0^2(2E + F)EFGx_0^3x_1 \\
&\quad + (a_0^2 + a_1^2)EFG^2x_0^2x_1^2 + (a_0Ex_0 - a_1Gx_1)^2EGx_0x_1
\end{aligned}
$$

Thus

$$
f_1f_2f_3 - f_1^2f_4 - f_3^2 > 0 \tag{D.4}
$$

According to (D.2), (D.3) and (D.4) the Routh-Hurwitz criteria are satisfied. Therefore, SS2 is stable as long as it exists.

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