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A model of a syntrophic relationship between two microbial species in a chemostat including maintenance

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8 Abstract

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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.

- 9 Keywords: Microbial ecosystems, Syntrophic relationship, Maintenance,
- ¹⁰ Stability, Food chains
- ¹¹ 2010 MSC: 34D20, 92D25, 92C45

12 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

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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 nutrient uptake 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

$$\begin{cases}
\frac{dS_{0}}{dt} = D\left(S_{0}^{in} - S_{0}\right) - f_{0}\left(\cdot\right)X_{0} \\
\frac{dX_{0}}{dt} = -\alpha DX_{0} + Y_{0}f_{0}\left(\cdot\right)X_{0} - a_{0}X_{0} \\
\frac{dS_{1}}{dt} = D\left(S_{1}^{in} - S_{1}\right) + \beta(1 - Y_{0})f_{0}\left(\cdot\right)X_{0} - f_{1}\left(\cdot\right)X_{1} \\
\frac{dX_{1}}{dt} = -\alpha DX_{1} + Y_{1}f_{1}\left(\cdot\right)X_{1} - a_{1}X_{1}
\end{cases}$$
(1)

where $Y_0 \leq 1$ and $Y_1 \leq 1$ are yield coefficients and $\beta \leq 1$ denotes an appropriate 17 constant. More precisely the terms $Y_0 f_0(\cdot) X_0$ and $Y_1 f_1(\cdot) X_1$ in the second and 18 fourth equations are the flux towards the biomasses X_0 and X_1 respectively. 19 The terms $(1 - Y_0)f_0(\cdot)X_0$ and $(1 - Y_1)f_1(\cdot)X_1$ represent the flux towards 20 other products that may or not be included in the model according to their 21 interactions with the dynamical variables. For instance, in the third equation, 22 $\beta(1-Y_0)f_0(\cdot)X_0$ is the part which goes to substrate S_1 and the rest goes to 23 other products which are left out in our model. Here, it is assumed that the units 24 of the state variable are well chosen. Mathematically we can perform a rescaling 25 of the variables, that is to say we change the units of the state variables, such 26 that the constant parameters Y_0 , Y_1 and $Y_2 = \beta(1 - Y_0)$ are fixed to 1, see (8) 27 and model (9) below. For a typical example, see model (21) in Section 6. 28

²⁹ Substrate S_0 and S_1 are introduced with an input concentration S_0^{in} and ³⁰ S_1^{in} respectively, and at dilution rate D. Depending on the technology used ³¹ to confine the reactions, the coefficient $\alpha \leq 1$ is not necessarily equal to 1 and ³² $1-\alpha$ represents the proportion of biomass which is retained in the reactor. This ³³ 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

 $_{43} f_0(\cdot) = f_0(S_0), f_1(\cdot) = f_1(S_1), (2)$

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

$$f_{52}$$
 $f_0(\cdot) = f_0(S_0, S_1), \quad f_1(\cdot) = f_1(S_1)$ (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, 30] and the more recent papers [10, 11, 19, 26, 31].

A model of a two-tiered microbial 'food chain' with feedback inhibition, which encapsulates the essence of the anaerobic digestion process was recently proposed [31]. 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 ex-64 cess sludge produced in wastewater treatment plants into more stable products 65 [17, 23]. One of its advantages is that the methane produced can be used prof-66 itably as a source of energy. It is usually considered that a number of metabolic 67 groups of microorganisms are involved sequentially in several serial and parallel conversion steps to finally produce methane and carbon dioxide. The Anaero-69 bic Digestion Model No. 1 (ADM1) of the IWA Task Group for Mathematical 70 Modelling of Anaerobic Digestion Processes [3, 12] is too complex to permit 71 mathematical analysis of its nonlinear dynamics and only numerical investiga-72 tions are available [7]. 73

The model of Xu et al. [31] includes maintenance terms and considers the 74 syntrophic associations between propionate degraders and methanogens. The 75 authors did not show that the non-trivial steady-state is necessarily stable. In 76 addition, simulation results, with the ADM1 consensus values, indicate that the 77 positive steady-state is always stable whenever it exists. For the operators of 78 anaerobic wastewater treatment systems the results of Xu et al. [31] show that 79 the syntrophic associations between propionate degraders and methanogens are 80 inherently stable under realistic environmental conditions. However, the possi-81 bility of an unstable positive steady-state was not excluded for other parameter 82 values and the title of [31], Maintenance affects the stability of a two-tiered mi-83 crobial 'food chain'? left unanswered the question of the effects of maintenance 84 from a more general viewpoint. In the present paper, we show that for any val-85 ues of the parameters the positive steady-state is stable as long as it exists, that 86 is to say, maintenance does not affect the stability of the considered two-tiered 87 microbial 'food chain', see [20]. 88

The paper is organized as follows. In Section 2, we review the different twostep 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. [31] 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.

98 2. Commensalism, mutualism and syntrophy

The different analyses of the class of models (1) available in the literature 99 essentially differ on the way the growth rate functions are characterized and 100 whether a specific input for S_1 or a coefficient α in the dilution rate of the 101 biomass is considered or not. In most cases, the models used are not generic 102 in the sense either model parameters are fixed or the growth functions are pre-103 defined (Monod, Haldane, etc). For details and informations on the various 104 models considered in the existing literature the reader can consult Table 2E 105 and Table 3 in the review paper [27]. Here we give a short review of the main 106 results which are related to our work. 107

Following Stephanopoulos [22] we say that 'Two populations of microorgan-108 isms which grow in a mixed culture and interact in such a way that one popula-109 tion (the commensal population) depends for its growth on the other population 110 and thus benefits from the interaction while the other population (the host) is 111 not affected by the growth of the commensal population constitutes an example 112 of commensalism'. Reilly [18] was the first to propose a mathematical study of a 113 pure commensalistic model (1,2), with $a_0 = a_1 = 0$ and $\alpha = 1$. He was interested 114 in explaining surprising oscillations observed within the course of an experiment 115 realized in making Saccharomyces carlsbergensis growing on fructose produced 116 by Acetobacter suboxyduns from mannitol. In particular, he established theo-117 retical conditions involving a feedback from the yeast to the bacteria. In this 118 study, explicit growth functions modelling the proposed feedback were used. 119

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 124 by Benyahia et al. [4]. Prior to these investigations, and regarding the potential 125 of anaerobic systems to produce renewable energy, the study of these models 126 were particularly important for optimizing anaerobic digestion, notably through 127 the synthesis of state observers and control feedback laws (cf. for instance [1, 2]). 128 Another fundamental ecological interactions which can be modelled by two-129 step reaction models with two microorganisms are mutualism and syntrophy. 130 Mutualism is defined as a situation where two organisms cooperate typically in 131 producing mutually the substrate necessary to the growth of the other [9]. A 132 syntrophic relationship between two organisms refers to growth functions of the 133 form (3) where the species exhibit mutualism but where, in contrast to what 134 happens in a purely symbiotic relationship, one of the species can grow without 135 the other. Important results of these studies were conditions under which a 136 stable coexistence may occur. Wilkinson et al. [30] studied the interactions in 137 a mixed bacterial population growing on methane. They considered the case of 138 growth functions of the form 139

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

where S_0 and S_1 are the dissolved oxygen and methanol concentrations respectively. The parameters m_i and K_i , i = 1, 2, are the classical Monod (or Michaelis-Menten) constants and L_1 is the methanol inhibition constant [see 30, Formulas (1) and (2) and Table V].

¹⁴⁵ Kreikenbohm and Bohl [14] considered the case where f_1 is a Monod function ¹⁴⁶ and the growth function f_0 takes the form

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

In this case, m_0 and K_0 are the classical Michaelis-Menten constants and K_1 acts as an inhibition constant related to the negative influence of the substrate S_1 on its own production. Moreover, the first organism is unable to grow unless the quotient S_1/S_0 is small enough, say, $S_1 < LS_0$, where L denotes an appropriate constant [see 14, Formulas (6a) and (7)].

¹⁵³ Burchard [8] extended the results of [14, 30] to a large class of more generic ¹⁵⁴ growth functions, including the special cases (4) and (5). He highlighted condi-¹⁵⁵ tions under which there is persistence or extinction. El Hajji et al. [10], moti-¹⁵⁶ vated by the analysis of the main studied steps of the anaerobic digestion where ¹⁵⁷ H_2 -producing acetogens are associated to H_2 -utilizing bacteria, considered the ¹⁵⁸ general case where the growth functions (3), satisfy the following properties:

$${}_{9} \qquad \qquad \frac{\partial f_0}{\partial S_0} > 0, \quad \frac{\partial f_0}{\partial S_1} < 0, \quad \frac{df_1}{dS_1} > 0 \tag{6}$$

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

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

Here, m_1 and K_1 are Michaelis-Menten-type constants, and L_0 is an inhibition 160 constant which represents the negative effect of S_0 on the growth of x_1 . The 161 mathematical analysis of this model showed the occurrence of bistability that 162 cannot be observed when $f_1(\cdot)$ depends only on S_0 . Sari et al. [19] considered 163 the general situation of a growth function $f_1(\cdot) = f_1(S_0, S_1)$, which is increasing 164 in S_1 and decreasing in S_0 and showed, in contrast with the case where $f_1(\cdot) =$ 165 $f_1(S_1)$ depends only on S_1 , that a multiplicity of positive equilibria can occur. 166 This work was motivated by the study of the influence of the presence of an input 167 term into the dynamics of S_1 again and by the consideration of more general 168 forms for growth rate functions to investigate the association of H_2 -producing 169 acetogens and H_2 -utilizing bacteria. Other models for which $f_0(\cdot) = f_0(S_0, S_1)$ 170 and $f_1(\cdot) = f_1(S_0, S_1)$, exhibiting the multiplicity of positive equilibria can be 171 found in [26]. 172

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, 30]. 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. [31] were the first to consider the

effects of maintenance terms in (1,3), in the particular case of the growth func-179 tions (4), and $S_1^{in} = 0$, $\alpha = 1$. As mentioned in the introduction these authors 180 were not able to show that the positive steady-state is stable if it exists. In 181 the present paper we will consider the general case (1,3) where growth func-182 tions satisfy (6) and with maintenance terms $(a_0 > 0, a_1 > 0)$ and $S_1^{in} = 0$, 183 $\alpha = 1$. We will prove that the positive steady-state is stable whenever it exists. 184 Therefore, in this paper we generalize [31] by allowing a larger class of growth 185 functions, we generalize [30] by allowing a larger class of growth functions and 186 maintenance terms, and we generalize [10] by allowing maintenance terms. For 187 the applications our results show that the syntrophic associations between pro-188 pionate degraders and methanogens are inherently stable for a wide range of 189 kinetics and whatever the parameters values are, not only for the kinetics (4) 190 and with the ADM1 consensus values of parameters as shown in [31]. 191

An important and interesting extension should be mentioned here: Weedermann et al. [28] 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. [29].

¹⁹⁷ 3. The model

In this paper, we study the model (1,3) with $\alpha = 1$ and $S_1^{in} = 0$. We use the notation $Y_2 = \beta(1 - Y_0)$. We obtain the following system

$$\begin{cases}
\frac{dS_0}{dt} = D\left(S_0^{in} - S_0\right) - f_0\left(S_0, S_1\right) X_0 \\
\frac{dX_0}{dt} = -DX_0 + Y_0 f_0\left(S_0, S_1\right) X_0 - a_0 X_0 \\
\frac{dS_1}{dt} = -DS_1 + Y_2 f_0\left(S_0, S_1\right) X_0 - f_1\left(S_1\right) X_1 \\
\frac{dX_1}{dt} = -DX_1 + Y_1 f_1\left(S_1\right) X_1 - a_1 X_1
\end{cases}$$
(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 $f_0(S_0, S_1)$ and $f_1(S_1)$:

204 **A1** For all $S_0 > 0$ and $S_1 \ge 0$, $f_0(S_0, S_1) > 0$ and $f_0(0, S_1) = 0$.

205 **A2** For all
$$S_1 > 0$$
, $f_1(S_1) > 0$ and $f_1(0) = 0$.

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

²⁰⁷ A4 For all
$$S_1 > 0$$
, $\frac{df_1}{dS_1}(S_1) > 0$.

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]:

$$s_0 = Y_2 S_0, \quad x_0 = \frac{Y_2}{Y_0} X_0, \quad s_1 = S_1, \quad x_1 = \frac{1}{Y_1} X_1,$$
 (8)

²¹⁸ We obtain the following system

$$\begin{cases} \frac{ds_0}{dt} = D(s_0^{in} - s_0) - \mu_0(s_0, s_1)x_0 \\ \frac{dx_0}{dt} = -Dx_0 + \mu_0(s_0, s_1)x_0 - a_0x_0 \\ \frac{ds_1}{dt} = -Ds_1 + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1 \\ \frac{dx_1}{dt} = -Dx_1 + \mu_1(s_1)x_1 - a_1x_1 \end{cases}$$

$$(9)$$

220 where $s_0^{in} = Y_2 S_0^{in}$ and μ_0 and μ_1 are defined by

$$\mu_0(s_0, s_1) = Y_0 f_0\left(\frac{1}{Y_2}s_0, s_1\right) \quad \text{and} \quad \mu_1(s_2) = Y_1 f_1(s_1) \tag{10}$$

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 (10) that functions μ_0 and μ_1 satisfy:

²²⁵ **H1** For all $s_0 > 0$ and $s_1 \ge 0$, $\mu_0(s_0, s_1) > 0$ and $\mu_0(0, s_1) = 0$.

²²⁶ H2 For all $s_1 > 0$, $\mu_1(S_1) > 0$ and $\mu_1(0) = 0$.

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

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

It should be noticed that (9) 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 (9) has non-negative components and is positively bounded and thus is defined for every positive t.

234 4. Steady-state and stability analysis

A steady-state of (9) is a solution of the following nonlinear algebraic system obtained from (9) by setting the right-hand sides equal to zero:

$$D(s_0^{in} - s_0) - \mu_0(s_0, s_1)x_0 = 0$$
(11)

$$-Dx_0 + \mu_0(s_0, s_1)x_0 - a_0x_0 = 0 \tag{12}$$

$$-Ds_1 + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1 = 0$$
(13)

$$-Dx_1 + \mu_1(s_1)x_1 - a_1x_1 = 0 \tag{14}$$

A steady-state exists (or is said to be 'meaningful' [31]) if and only if all its components are non-negative. From equation (12) we deduce that:

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$$x_0 = 0$$
 or $\mu_0(s_0, s_1) = D + a_0$ (15)

 $_{240}$ and from equation (14) we deduce that:

$$x_1 = 0 \quad \text{or} \quad \mu_1(s_1) = D + a_1$$
 (16)

The case $x_0 = 0$ and $x_1 > 0$ is excluded. Indeed, as a consequence of (16), we have $\mu_1(s_1) = D + a_1$ and, as a consequence of (13), we have $Ds_1 + (D + a_1)x_1 =$ 0, which is impossible since $s_1 \ge 0$ and $x_1 > 0$. Therefore, three cases must be distinguished:

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.

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.

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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 $s_1 \ge 0$ and $y \in [0, \sup \mu_1(\cdot))$

$$s_2 \qquad s_1 = M_1(y) \Longleftrightarrow y = \mu_1(s_1) \tag{17}$$

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 $s_0, s_1 \ge 0$, and $y \in [0, \sup \mu_0(\cdot, s_1))$

$$s_0 = M_0(y, s_1) \Longleftrightarrow y = \mu_0(s_0, s_1) \tag{18}$$

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 (23) considered in Section 6, see formulas (25,(26)). We define the functions:

$$F_0(D) = M_0(D + a_0, 0)$$

$$F_1(D) = M_1(D + a_1) + M_0(D + a_0, M_1(D + a_1))$$
(19)

Notice that $F_1(D) > F_0(D)$ for all $D \ge 0$, 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 (9).

Proposition 1. Assume that assumptions H1-H4 hold. Then (9) has at most
three steady-states:

•
$$SS0 = (s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0)$$

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.

²⁷³ The proof is given in the Appendix.

Notice that SS1 exists as soon as SS0 becomes unstable and SS2 exists 274 as soon as SS1 becomes unstable. One concludes that for any value of the 275 operating parameters, there is always one, and only one, steady-state which is 276 stable. The results are summarized in Table 1. When decay effects are not taken 277 into account, i.e. $a_0 = a_1 = 0$, the system can be reduced to a planar system 278 and global stability results can be obtained [10, 19]: for any pair of operating 279 parameters, there is always one, and only one, steady-state which is globally 280 asymptotically stable. 281



Figure 1: Operating diagram without (a) and with (b) maintenance effects. The values u_0 and u_1 are defined by (20)

282 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

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

by (19). 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 dashed curve in 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 solid curve in 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:

$$u_0 = F_0(0) = M_0(a_0, 0), \ u_1 = F_1(0) = M_1(a_1) + M_0(a_0, M_1(a_1)), \quad (20)$$

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 $a_0 \geq \sup_{s_0>0} \mu_0(s_0 - M_1(a_1), M_1(a_1))$, $F_1(0)$ 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

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

²⁹⁵ 6. A two-tiered microbial 'food chain'

The model considered in [31] involves a two-tiered microbial 'food chain' with feedback inhibition, consisting of a propionate degrader and a hydrogenotrophic

²⁹⁸ methanogen. The propionate degrader produces hydrogen which inhibits its own

²⁹⁹ growth. Using the notations of ADM1 the model can be written as

300

$$\begin{cases} \frac{dS_{pro}}{dt} = D\left(S_{pro,in} - S_{pro}\right) - f_{0}\left(S_{pro}, S_{H_{2}}\right) X_{pro} \\ \frac{dX_{pro}}{dt} = -DX_{pro} + Y_{pro}f_{0}\left(S_{pro}, S_{H_{2}}\right) X_{pro} - k_{dec,pro}X_{pro} \\ \frac{dS_{H_{2}}}{dt} = -DS_{H_{2}} + \beta\left(1 - Y_{pro}\right) f_{0}\left(S_{pro}, S_{H_{2}}\right) X_{pro} - f_{1}\left(S_{H_{2}}\right) X_{H_{2}} \\ \frac{dX_{H_{2}}}{dt} = -DX_{H_{2}} + Y_{H_{2}}f_{1}\left(S_{H_{2}}\right) X_{H_{2}} - k_{dec,H_{2}}X_{H_{2}} \end{cases}$$
(21)

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 yield coefficients and $\beta = 0.43$ represents the part which goes to hydrogen substrate. The rest $(1 - \beta = 0.57)$ goes to acetate which is left out in the model. Both growth functions take Monod form with an hydrogen inhibition for the first one

$$f_0(S_{pro}, S_{H_2}) = \frac{k_{m, pro} S_{pro}}{K_{s, pro} + S_{pro}} \frac{1}{1 + \frac{S_{H_2}}{K_{I, H_2}}}, \quad f_1(S_{H_2}) = \frac{k_{m, H_2} S_{H_2}}{K_{s, H_2} + S_{H_2}}$$
(22)

Here, apart from the two operating (or control) parameters, which are the in-307 flowing propionate concentration $S_{pro,in}$ and the dilution rate D, that can vary, 308 all others have biological meaning and are fixed depending on the organisms and 309 substrate considered [see 31, Table 1]. The aim of Xu et al. [31] was to study 310 the stability of the steady-states of the model (21,22) while varying the two op-311 erating (or control) parameters D and $S_{pro,in}$. The system (21,22) can have at 312 most three steady-states: a trivial solution where both populations are washed 313 out (SS0), a solution where X_{H_2} is washed out while X_{pro} survives (SS1) and 314 a positive solution where both populations survive (SS2). The local stability of 315 each steady-state was tested by linearisation around the steady-state values of 316 the variables. 317

The basic results of the analysis of [31] 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 (21), 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 (21), the authors were not able to check all the Routh-Hurwitz criteria for 323 SS2. They claimed that SS2 is not necessarily stable in theory when it exists 324 and they established numerically that with the ADM1 parameters values, SS2 325 is stable as long as it exists. However they did not give any values for the 326 biological parameters for which, under some operating parameters, SS2 becomes 327 unstable. As a consequence of Proposition 1, we can say that, for all values of 328 the parameters, SS2 is stable whenever it exits, which actually gives an answer 320 to the questions asked by [31] in their paper. 330

More precisely, using the following simplified notations in (21)

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

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

and using the rescaling (10) and the biological parameters in (22) we obtain the model (9) with the following growth function:

$$\mu_0(s_0, s_1) = \frac{m_0 s_0}{K_0 + s_0} \frac{1}{1 + s_1/K_i}, \quad \mu_1(s_1) = \frac{m_1 s_1}{K_1 + s_1}$$
(23)

334 where

335

$$m_0 = Y_0 k_{m,pro}, \quad K_0 = Y_2 K_{s,pro}, \quad K_i = K_{I,H_2}$$

$$m_1 = Y_1 k_{m,H_2}, \quad K_1 = K_{s,H_2}$$
(24)

Let us describe our results in the particular case (9,23). Notice that the growth functions (23) 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

₃₄₀
$$y \in [0, m_1) \mapsto M_1(y) = \frac{K_1 y}{m_1 - y},$$
 (25)

$$_{341} \qquad y \in \left[0, \frac{m_1}{1 + s_1/K_i}\right) \mapsto M_0(y, s_1) = \frac{K_0 y}{\frac{m_0}{1 + s_1/K_i} - y} \tag{26}$$

Therefore, the functions
$$F_1(D)$$
 and $F_2(D)$ defined by (19) are given explicitly

343 by

$$F_{0}(D) = \frac{K_{0}(D+a_{0})}{m_{1}-D-a_{0}}$$

$$F_{1}(D) = \frac{K_{1}(D+a_{1})}{m_{1}-D-a_{1}} + \frac{K_{0}(D+a_{0})}{\frac{m_{0}}{1+\frac{K_{1}(D+a_{1})}{(m_{1}-D-a_{1})K_{i}}} - D-a_{0}}$$
(27)

344

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:

$$m_0 s_0 = (D + a_0)(K_0 + s_0) \left(1 + \frac{s_0^{in} - s_0}{K_i}\right)$$
(28)

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

Proposition 2. Assume that μ_0 and μ_1 are given by (23). Let $F_0(D)$ and $F_1(D)$ be defined by (27). Then (9) has at most three steady-states

•
$$SS0 = (s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0)$$

It always exists. It is stable if and only if $s_0^{in} < F_0(D)$

It always exists. It is stable if and only if
$$s_0^{so} < F_0(D)$$
.

•
$$SS1 = \left(s_0, x_0 = \frac{D}{D+a_0} \left(s_0^{in} - s_0\right), s_1 = s_0^{in} - s_0, x_1 = 0\right)$$

where s_0 is the positive solution of the quadratic equation (28). 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
 $s_1 = \frac{K_1(D+a_1)}{m_1 - D - a_1}, \qquad s_0 = \frac{K_0(D+a_0)}{\frac{m_0}{1 + \frac{s_1}{V}} - D - a_0}$

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

358

As a consequence of this result we obtain the results of [31], where the stability of SS2 was proved only for a particular set of parameter values. To make the comparison possible the reader is advised on the main difference between our

³⁶² approach and [31]: we use the rescaling (10) and hence work with the growth

³⁶³ functions (23), while Xu et al. [31] use a dimensionless rescaling. Despite this ³⁶⁴ difference, both approaches are equivalent and hence must give the same re-³⁶⁵ sults. Our quadratic equation (28) used in the description of SS1 is the same ³⁶⁶ as their quadratic equation (A.1), or the quadratic equation without numbering ³⁶⁷ preceding equation (B.1) [see 31, Appendix A and B].

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

Table 3: Nominal parameters values.



Figure 2: Operating diagram of the model (9)-(23). (a) The model was parametrised with the ADM1 consensus values listed in Table 3. (b) A magnification showing the values $u_0 = 4.96 \ 10^{-3}$, $u_1 = 5.31 \ 10^{-3}$ defined by (20).

For the numerical simulations we will use the nominal values of Table 3 obtained from Table 1 of [31] by using the formulas (24) 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 [31], since these authors used another rescaling.

373 7. Discussion

Following [31], we considered a two-tiered 'food chain' with feedback inhi-374 bition, which is a generalized model describing the syntrophic interaction of 375 a propionate degrader and a hydrogenotropic methanogen. In the absence of 376 maintenance these authors proved that this two-tiered 'food chain' is always sta-377 ble. When maintenance is included in the model they were not able to check the 378 Routh-Hurwitz criteria, and since the possibility of having at least one pair of 379 complex eigenvalues with positive real parts is not theoretically excluded, they 380 concluded that Hopf bifurcation can originate from SS2 [see 31, Appendix B]. 381 However, using the consensus parameters of ADM1 and numerical simulations, 382 they have shown that the model of the methanogenic two-tiered propionate-383 hydrogen food chain is always stable [see 31, Section 6.2]. In this work we have 384 generalized the model of the two-tiered 'food chain' of [31] by considering generic growth functions and we established the stability of the generalized model with 386 maintenance terms. 387

In [31], the authors point out that introducing decay or maintenance in 388 the classical predator-prey models results in instability and chaos [13]. For 389 more details on food-chains in the chemostat the reader may consult [6, 24, 25]. 390 Therefore, they observed that, in spite of the fundamental differences between 391 their 'food chain' and the classical predator-prey models, the same intrinsic 302 effect of maintenance on the stability of the food chain is observed [see 31, 393 Section 7]: When maintenance is included in its description, the two-tiered 394 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 396 always stable, so that it is fundamentally different from the classical predator-397 prey model. 398

It should be noticed that the rescaling used by [31] gives a dimensionless model. However, our present rescaling (10) does not give a dimensionless model.

The new variables s_0 , x_0 , s_1 and x_1 have the same dimensions as the original 401 variables S_0 , X_0 , S_1 and X_1 . The original growth functions (22) are trans-402 formed by our rescaling (10) in the growth functions (23) with the same scale 403 imbalance in the half-saturation rates, see Table 3. We cannot benefit from the 404 dimensionless rescaling used by Xu et al. [31], because this rescaling uses some 405 kinetics parameters of the specific growth functions (22) while we work with 406 general unspecified growth functions. The benefit of our rescaling (10) is that 407 it permits to fix the constant yields parameters Y_0 , Y_1 and Y_2 in (7) to 1, as 408 shown by the rescaled model (9). 409

We were successful in checking the Routh-Hurwitz criteria because we work 410 with general growth functions (defined by their qualitative properties given in 411 assumptions A1-A4) and our computations are not encumbered by the specific 412 form of the growth functions considered by Xu et al. [31]. These authors noticed 413 [see 31, Section 7] that direct application of symbolic analysis programs, such 414 as Maple or Mathematica, did not provide adequate solutions for the stability 415 of the system. Actually we used the symbolic analysis program Maple to verify 416 that the coefficients β_i in the expression of the term $f_1 f_2 f_3 - f_1^2 f_4 - f_3^2$ given 417 in Appendix D are correct. It should be noticed that [31] have claimed [see 31, 418 Remark 1] that their method is still effective for other growth functions. Our 419 main contribution was to believe them and to try to solve the problem with 420 general growth functions. 421

In the model (7) considered in this work, the first species X_0 uses the sub-422 strate S_0 for its growth and produces a substrate S_1 consumed by the second 423 species X_1 for its growth. The substrate S_1 produced by the first species in-424 hibits its own growth, that is, the growth function $f_0(S_0, S_1)$ is decreasing with 425 respect to S_1 . In practice, and in many complex models as the ADM1, it hap-426 pens that the second species is also inhibited by the first substrate. Thus, it 427 is interesting to consider the case where the second species is inhibited by the 428 substrate S_0 , namely that $f_1(S_0, S_1)$ also depends on S_0 and is decreasing with 429 respect to S_0 . It has been shown by Sari et al. [19] that the introduction of this 430 last inhibiting relationship in the model completely changes the model proper-431

ties 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.

441 Appendix A. Stability analysis

We give the proof of Prop. 1. A steady-state (s_0, x_0, s_1, x_1) of (9) is a solution of the set of algebraic equations (11-14). 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 (9). 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:

$${}_{448} \qquad J = \begin{bmatrix} -D - Ex_0 & -\mu_0 & Fx_0 & 0 \\ Ex_0 & \mu_0 - D - a_0 & -Fx_0 & 0 \\ Ex_0 & \mu_0 & -D - Fx_0 - Gx_1 & -\mu_1 \\ 0 & 0 & Gx_1 & \mu_1 - D - a_1 \end{bmatrix}$$
(A.1)

where

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

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.

453 Appendix B. SS0

At SS0, $x_0 = 0$, $x_1 = 0$. As a result of (11) and (13), $s_0 = s_0^{in}$ and $s_1 = 0$. SS0 always exists. Evaluated at SS0, the Jacobian matrix (A.1) becomes

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

454 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 (18) we have the following equivalence

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

⁴⁵⁷ Therefore, according to (19), (B.1) is equivalent to $s_0^{in} > F_0(D)$.

458 Appendix C. SS1

At SS1, $x_0 \neq 0$, $x_1 = 0$. As a consequence of (15) $\mu_0(s_0, s_1) = D + a_0$. As a result of (11) and (13)

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

Hence $x_0 = \frac{D}{D+a_0} (s_0^{in} - s_0)$ 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:

$$\lambda^{2} + [D + (E + F)x_{0}]\lambda + (D + a_{0})(E + F)x_{0} = 0$$

462 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 $\lambda_1 < 0$.

⁴⁶⁴ Therefore SS1 is stable if and only if

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

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

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

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),

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

⁴⁶⁶ Therefore, the condition (C.2) of stability of SS1 is equivalent to:

467
$$D + a_0 < \mu_0 \left(s_0^{in} - M_1(D + a_1), M_1(D + a_1) \right)$$
 (C.3)

Since the function $s_0 \mapsto \mu_0(s_0, M_1(D + a_1))$ is increasing, and using (18), 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 (19), equivalent to

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

468 Appendix D. SS2

At SS2, $x_0 \neq 0$, $x_1 \neq 0$. As a consequence of (15) and (16) s_0 and s_1 are solutions of the set of equations

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

469 Using (17) we obtain $s_1 = M_1(D + a_1)$ and s_0 is a solution of equation

470

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

Using (18) we obtain $s_0 = M_0 (D + a_0, M_1 (D + a_1))$. As a result of (11) and (13)

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

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

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

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

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

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

471 Hence

472

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

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

$$f_1 f_2 - f_3 = 2D^3 + \alpha_2 D^2 + \alpha_1 D + \alpha_0$$

where

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

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

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

473 Thus

474

$$f_1 f_2 - f_3 > 0 \tag{D.3}$$

On the other hand we have

$$f_1 f_2 f_3 - f_1^2 f_4 - f_3^2 = \beta_5 D^5 + \beta_4 D^4 + \beta_3 D^3 + \beta_2 D^2 + \beta_1 D + \beta_0$$

where

$$\beta_5 = 2(Hx_0 + Gx_1)$$
$$\beta_4 = 4(Hx_0 + Gx_1)^2 + 2a_0Hx_0 + 2a_1Gx_1$$

475

$$\beta_3 = 2(Hx_0 + Gx_1)^3 + 4EG(Hx_0 + Gx_1)x_0x_1 + 5a_0H^2x_0^2 + (a_0 + a_1)(3E + 5F)Gx_0x_1 + 5a_1G^2x_1^2$$

476

$$\beta_{2} = 4EG(Hx_{0} + Gx_{1})^{2}x_{0}x_{1}$$

$$+3a_{0}H^{3}x_{0}^{3} + (a_{0}E + 2a_{1}E + 6a_{0}H + 3a_{1}F)GHx_{0}^{2}x_{1}$$

$$+(2a_{0}E + a_{1}E + 3a_{0}F + 6a_{1}H)G^{2}x_{0}x_{1}^{2} + 3a_{1}G^{3}x_{1}^{3}$$

$$+a_{0}^{2}F(F + 2E)x_{0}^{2} + (a_{0}Ex_{0} - a_{1}Gx_{1})^{2} + 2a_{0}a_{1}GFx_{0}x_{1}$$

477

$$\beta_{1} = 2E^{2}G^{2}(Hx_{0} + Gx_{1})x_{0}^{2}x_{1}^{2} + (4a_{0} + a_{1})EGH^{2}x_{0}^{3}x_{1}$$

$$+ (a_{0} + a_{1})(3E + 5F)EG^{2}x_{0}^{2}x_{1}^{2} + (a_{0} + 4a_{1})EG^{3}x_{0}x_{1}^{3}$$

$$+ a_{0}^{2}(3E^{2} + 3EF + F^{2})Fx_{0}^{3} + a_{0}(2a_{0}E + a_{0}F + 2a_{1}F)GFx_{0}^{2}x_{1}$$

$$+ (Ex_{0} + Gx_{1})(a_{0}Ex_{0} - a_{1}Gx_{1})^{2} + (2a_{0}a_{1} + a_{1}^{2})G^{2}Fx_{0}x_{1}^{2}$$

478

$$\beta_0 = (a_0 + a_1)E^2G^2(Hx_0 + Gx_1)x_0^2x_1^2 + a_0^2(2E + F)EFGx_0^3x_1 + (a_0^2 + a_1^2)EFG^2x_0^2x_1^2 + (a_0Ex_0 - a_1Gx_1)^2EGx_0x_1$$

479 Thus

$$f_{1}f_{2}f_{3} - f_{1}^{2}f_{4} - f_{3}^{2} > 0 \tag{D.4}$$

⁴⁸¹ According to (D.2), (D.3) and (D.4) the Routh-Hurwitz criteria are satisfied.
⁴⁸² Therefore, SS2 is stable as long as it exists.

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491 References

- [1] V. Alcaraz-Gonzalez, J. Harmand, A. Rapaport, J. P. Steyer, V. Gonzalez
 Alvarez, C. Pelayo Ortiz (2002). Software sensors for highly uncertain
 WWTPs : a new approach based on interval observers. Water Research
 36, 2515-2524. doi:10.1016/S0043-1354(01)00466-3
- ⁴⁹⁶ [2] V. Alcaraz-Gonzalez, J. Harmand, A. Rapaport, J. P. Steyer, V. Gonzalez
 ⁴⁹⁷ Alvarez, C. Pelayo Ortiz (2005). Application of a Robust Interval Observer
 ⁴⁹⁸ to an Anaerobic Digestion Process. Developments in Chemical Engineering
 ⁴⁹⁹ and Mineral Processing 13, 3-4. DOI: 10.1002/apj.5500130308
- [3] D.J. Batstone, J. Keller, I. Angelidaki, S.V. Kalyuzhnyi, S.G. Pavlostathis,
 A. Rozzi, W.T.M Sanders, H. Siegrist, V.A. Vavilin (2002). The Iwa Anaer obic Digestion Model No 1 (ADM1). Water Sci. Technol. 45, 65-73.
- [4] B. Benyahia, T. Sari, B. Cherki, J. Harmand (2012). Bifurcation and stability analysis of a two step model for monitoring anaerobic digestion processes. J. Process Control 22, 1008-1019. doi:10.1016/j.jprocont.2012.04.012
- [5] O. Bernard, Z. Hadj-Sadock, D. Dochain, A. Genovesi, J.-P. Steyer (2001).
 Dynamical model development and parameter identification for an anaer obic wastewater treatment process. Biotechnol. Bioeng. 75, 424-438. DOI:
 10.1002/bit.10036
- [6] M.P. Boer, B.W. Kooi, S.A.L.M. Kooijman (1998). Food chain dynamics
 in the chemostat. Mathematical Biosciences 150, 43-62. doi:10.1016/S0025 5564(98)00010-8
- [7] A. Bornhöft, R. Hanke-Rauschenbach, K. Sundmacher (2013). Steadystate analysis of the Anaerobic Digestion Model No. 1 (ADM1). Nonlinear
 Dynamics 73, 535-549. DOI 10.1007/s11071-013-0807-x
- [8] A. Burchard (1994). Substrate degradation by a mutualistic association of two species in the chemostat. J. Math. Biol. 32, 465–489. DOI
 10.1007/BF00160169

- [9] M. El Hajji, J. Harmand, H. Chaker and C. Lobry (2009). Association 519 between competition and obligate mutualism in a chemostat, J. Biol. Dy-520 namics, 3, 635-647. DOI:10.1080/17513750902915978 521
- [10] M. El Hajji, F. Mazenc and J. Harmand (2010). A mathematical study of 522 a syntrophic relationship of a model of anaerobic digestion process. Math. 523 Biosci. Eng. 7, 641-656. doi: 10.3934/mbe.2010.7.641

524

- [11] E. Harvey, J. Heys, T. Gedeon (2014). Quantifying the effects of the 525 division of labor in metabolic pathways. J. Theoret. Biol. 360, 222-242. 526 doi:10.1016/j.jtbi.2014.07.011 527
- [12] IWA Task Group for Mathematical Modelling of Anaerobic Digestion Pro-528 cesses (2002). Anaerobic Digestion Model No.1 (ADM1). Scientific and 529 Technical Report No. 13, IWA Publishing, London. 530
- [13] B.W. Kooi, M.P. Boer (2003). Chaotic behaviour of a predator-prey sys-531 tem in the chemostat. Second International Conference on Dynamics of 532 Continuous, Discrete and Impulsive Systems (London, ON, 2001). Dyn. 533 Contin. Discrete Impuls. Syst. Ser. B Appl. Algorithms 10, 259-272. 534
- [14] R. Kreikenbohm, E. Bohl (1986). A mathematical model of syn-535 trophic cocultures in the chemostat. FEMS Microbiol. Ecol. 38: 131-140. 536 doi:10.1016/0378-1097(86)90044-3 537
- [15] R. Kreikenbohm, E. Bohl (1988). Bistability in the Chemostat. Ecological 538 Modelling 43, 287-301. doi:10.1016/0304-3800(88)90009-9 539
- [16] B.J. Ni, G.P. Sheng, Yu, H.Q. (2011). Model-based characterization of 540 endogenous maintenance, cell death and predation processes of activated 541 sludge in sequencing batch reactors. Chemical Engineering Science 66, 747-542 754. doi:10.1016/j.ces.2010.11.033 543
- [17] I. Ramirez, E.I.P. Volcke, R. Rajinikanth, J.P. Steyer (2009). Modelling 544
- microbial diversity in anaerobic digestion through an extended adm1 model. 545
- Water Res. 43, 2787-2800. doi:10.1016/j.watres.2009.03.034 546

- ⁵⁴⁷ [18] P.J. Reilly (1974). Stability of commensalistic systems. Biotechnol. Bioeng.
 ⁵⁴⁸ 16, 1373-1392. DOI: 10.1002/bit.260161006
- ⁵⁴⁹ [19] T. Sari, M. El Hajji, J. Harmand (2012). The mathematical analysis of
 ⁵⁵⁰ a syntrophic relationship between two microbial species in a chemostat.
- ⁵⁵¹ Math. Biosci. Eng. 9, 627-645. doi:10.3934/mbe.2012.9.627
- ⁵⁵² [20] T. Sari, J. Harmand (2014). Maintenance does not affect the stability
 ⁵⁵³ of a two-tiered microbial 'food chain'. https://hal.archives-ouvertes.fr/hal⁵⁵⁴ 01026149v1
- M. Sbarciog, M. Loccufier, E Noldus (2010). Determination of appropriate operating strategies for anaerobic digestion systems. Biochemical
 engineering journal 51 (3), 180-188. doi:10.1016/j.bej.2010.06.016
- ⁵⁵⁸ [22] G. Stephanopoulos (1981). The dynamics of commensalism. Biotechnol.
 ⁵⁵⁹ Bioeng. 23, 2243-2255. DOI:10.1002/bit.260231008
- J.B. Van Lier, N.A. Mahmoud, G. Zeeman (2008). Anaerobic wastewater treatment. In: M. Henze, M.C.M. van Loodsdrecht, G.A. Ekema, D.
 Brdjanovic (Eds.), Biological Wastewater Treatment: Principle, Modelling
 and Design. IWA Publishing, London, 415-456.
- G.A.K. van Voorn, B.W. Kooi, M.P. Boer (2010). Ecological consequences
 of global bifurcations in some food chain models. Mathematical Biosciences
 226, 120-133. doi:10.1016/j.mbs.2010.04.005
- ⁵⁶⁷ [25] D.V. Vayenas, S. Pavlou (1999). Chaotic dynamics of a food web in
 a chemostat. Mathematical Biosciences 162, 69-84. doi:10.1016/S0025 ⁵⁶⁹ 5564(99)00044-9
- E.I.P. Volcke, M. Sbarciog, E.J.L. Noldus, B. De Baets, M. Loccufier (2010). Steady-state multiplicity of two-step biological conversion
 systems with general kinetics. Mathematical Biosciences 228, 160-170.
 doi:10.1016/j.mbs.2010.09.004

- ⁵⁷⁴ [27] M.J. Wade, J. Harmand, B. Benyahia, T. Bouchez, S. Chaillou, B. Cloez,
 ⁵⁷⁵ J.-J. Godon, B. Moussa Boudjemaa, A. Rapaport, T. Sari, R. Arditi, C. Lo⁵⁷⁶ bry (2016). Perspectives in mathematical modelling for microbial ecology.
- 577 Ecological Modelling 321, 64-74. doi:10.1016/j.ecolmodel.2015.11.002
- M. Weedermann, G. Seo, G. Wolkowicz (2013). Mathematical Model of Anaerobic Digestion in a Chemostat: Effects of Syntrophy and Inhibition. Journal of Biological Dynamics 7, 59-85.
 DOI:10.1080/17513758.2012.755573
- [29] M. Weedermann, G. Wolkowicz, J. Sasara (2015). Optimal biogas produc tion in a model for anaerobic digestion. Nonlinear Dynamics 81, 1097-1112.
 DOI 10.1007/s11071-015-2051-z
- ⁵⁸⁵ [30] T. G. Wilkinson, H. H. Topiwala, G. Hamer (1974). Interactions in a
 ⁵⁸⁶ Mixed Bacterial Population Growing on Methane in Continuous Culture.
 ⁵⁸⁷ Biotechnol. Bioeng. 16, 41-59. DOI: 10.1002/bit.260160105
- [31] A. Xu, J. Dolfing, T.P. Curtis, G. Montague, E. Martin (2011). Maintenance affects the stability of a two-tiered microbial 'food chain'? J.
 Theoret. Biol. 276, 35-41. doi:10.1016/j.jtbi.2011.01.026