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

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8 **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.

9 *Keywords:* Microbial ecosystems, Syntrophic relationship, Maintenance,
10 Stability, Food chains

11 *2010 MSC:* 34D20, 92D25, 92C45

12 **1. Introduction**

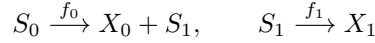
Two-step models are commonly used to describe microbial systems, which take the form of a cascade of two biological reactions where one substrate S_0

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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:



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

$$16 \quad \begin{cases} \frac{dS_0}{dt} = D(S_0^{in} - S_0) - f_0(\cdot)X_0 \\ \frac{dX_0}{dt} = -\alpha DX_0 + Y_0 f_0(\cdot)X_0 - a_0 X_0 \\ \frac{dS_1}{dt} = D(S_1^{in} - S_1) + \beta(1 - Y_0)f_0(\cdot)X_0 - f_1(\cdot)X_1 \\ \frac{dX_1}{dt} = -\alpha DX_1 + Y_1 f_1(\cdot)X_1 - a_1 X_1 \end{cases} \quad (1)$$

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

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

34 its most general assertion, is the consumption of energy for all processes other
35 than growth: it is modelled either by adding a negative term on the substrate
36 dynamic without associating it to growth or by considering a decay term on
37 the biomass dynamics, as in (1). For more information about the modelling of
38 maintenance, the reader is referred to [16]. These models present the advantage
39 of being complex enough to capture important process properties while being
40 simple enough to be mathematically studied.

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

$$43 \quad f_0(\cdot) = f_0(S_0), \quad f_1(\cdot) = f_1(S_1), \quad (2)$$

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

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

$$52 \quad f_0(\cdot) = f_0(S_0, S_1), \quad f_1(\cdot) = f_1(S_1) \quad (3)$$

53 the system is known as syntrophic. For instance if the first organism is inhibited
54 by high concentrations of the product S_1 , the extent to which the substrate S_0
55 is degraded by the organism X_0 depends on the efficiency of the removal of the
56 product S_1 by the bacteria X_1 . The mathematical analysis of such model is
57 more delicate than commensalistic models, see for instance [8, 14, 15, 30] and
58 the more recent papers [10, 11, 19, 26, 31].

59 A model of a two-tiered microbial ‘food chain’ with feedback inhibition,
60 which encapsulates the essence of the anaerobic digestion process was recently
61 proposed [31]. Anaerobic digestion is a biological process that converts organic
62 matter into a gaseous mixture composed mainly of methane and carbon diox-
63 ide through the action of a complex bacterial and archaeal ecosystem. It is

64 often used for the treatment of concentrated wastewaters or to convert the ex-
65 cess sludge produced in wastewater treatment plants into more stable products
66 [17, 23]. One of its advantages is that the methane produced can be used prof-
67 itably as a source of energy. It is usually considered that a number of metabolic
68 groups of microorganisms are involved sequentially in several serial and parallel
69 conversion steps to finally produce methane and carbon dioxide. The Anaero-
70 bic Digestion Model No. 1 (ADM1) of the IWA Task Group for Mathematical
71 Modelling of Anaerobic Digestion Processes [3, 12] is too complex to permit
72 mathematical analysis of its nonlinear dynamics and only numerical investiga-
73 tions are available [7].

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

89 The paper is organized as follows. In Section 2, we review the different two-
90 step models that have been proposed in the literature and we recall what useful
91 informations were obtained for applications from their qualitative mathematical
92 analysis. In Section 3 we present the hypothesis on (1,3). In Section 4 we give
93 the description of the steady-state and their stability. In Section 5 we describe
94 the operating diagram. In Section 6 we apply our results to the model of Xu et

95 al. [31] and we give an answer to open questions on the stability of the positive
96 steady-state of their model. Concluding remarks are given in Section 7. The
97 technical proofs of the results are given in the Appendix.

98 **2. Commensalism, mutualism and syntrophy**

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

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

120 An important contribution on the modelling of anaerobic digestion as a
121 commensalistic system is the model by Bernard et al. [5]. The authors considered
122 a Monod function for f_0 and a Haldane function for f_1 . Sbarciog et al. [21]
123 studied this model for $\alpha = 1$ while the interesting case where $0 < \alpha < 1$ and

124 where growth functions were characterized by qualitative properties was studied
 125 by Benyahia et al. [4]. Prior to these investigations, and regarding the potential
 126 of anaerobic systems to produce renewable energy, the study of these models
 127 were particularly important for optimizing anaerobic digestion, notably through
 128 the synthesis of state observers and control feedback laws (cf. for instance [1, 2]).

129 Another fundamental ecological interactions which can be modelled by two-
 130 step reaction models with two microorganisms are mutualism and syntrophy.
 131 Mutualism is defined as a situation where two organisms cooperate typically in
 132 producing mutually the substrate necessary to the growth of the other [9]. A
 133 syntrophic relationship between two organisms refers to growth functions of the
 134 form (3) where the species exhibit mutualism but where, in contrast to what
 135 happens in a purely symbiotic relationship, one of the species can grow without
 136 the other. Important results of these studies were conditions under which a
 137 stable coexistence may occur. Wilkinson et al. [30] studied the interactions in
 138 a mixed bacterial population growing on methane. They considered the case of
 139 growth functions of the form

$$140 \quad f_0(S_0, S_1) = \frac{m_0 S_0}{K_0 + S_0} \frac{1}{1 + S_1/L_1}, \quad f_1(S_1) = \frac{m_1 S_1}{K_1 + S_1} \quad (4)$$

141 where S_0 and S_1 are the dissolved oxygen and methanol concentrations re-
 142 spectively. The parameters m_i and K_i , $i = 1, 2$, are the classical Monod (or
 143 Michaelis-Menten) constants and L_1 is the methanol inhibition constant [see
 144 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

$$147 \quad f_0(S_0, S_1) = \begin{cases} \frac{m_0(S_0 - S_1/L)}{K_0 + S_0 + K_1 S_1} & \text{if } S_0 - S_1/L > 0 \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

148 In this case, m_0 and K_0 are the classical Michaelis-Menten constants and K_1 acts
 149 as an inhibition constant related to the negative influence of the substrate S_1 on
 150 its own production. Moreover, the first organism is unable to grow unless the
 151 quotient S_1/S_0 is small enough, say, $S_1 < LS_0$, where L denotes an appropriate
 152 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:

$$159 \quad \frac{\partial f_0}{\partial S_0} > 0, \quad \frac{\partial f_0}{\partial S_1} < 0, \quad \frac{df_1}{dS_1} > 0 \quad (6)$$

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

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

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

173 All these studies do not include maintenance terms. This short review of the
174 existing literature shows that under conditions like (6) and without maintenance
175 terms ($a_0 = a_1 = 0$), the positive steady-state is unique and stable, if it exists
176 [8, 10, 14, 30]. On the other hand as soon as $f_1(\cdot) = f_1(S_0, S_1)$ may depend on
177 S_0 then instability of the positive steady-state can occur [15, 19, 26].

178 To the best of our knowledge, Xu et al. [31] were the first to consider the

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

192 An important and interesting extension should be mentioned here: Weeder-
 193 mann et al. [28] proposed an 8-dimensional mathematical model, which includes
 194 syntrophy and inhibition, both mechanisms considered by Bernard et al. [5] and
 195 by El Hajji et al. [10]. The effects of maintenance terms are considered by
 196 Weedermann et al. [29].

197 3. The model

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

$$200 \left\{ \begin{array}{l} \frac{dS_0}{dt} = D(S_0^{in} - S_0) - f_0(S_0, S_1) X_0 \\ \frac{dX_0}{dt} = -DX_0 + Y_0 f_0(S_0, S_1) X_0 - a_0 X_0 \\ \frac{dS_1}{dt} = -DS_1 + Y_2 f_0(S_0, S_1) X_0 - f_1(S_1) X_1 \\ \frac{dX_1}{dt} = -DX_1 + Y_1 f_1(S_1) X_1 - a_1 X_1 \end{array} \right. \quad (7)$$

201 Notice that we do not assume any specific analytical expression for the growth
 202 and inhibition functions. Our analysis will use only the following general as-
 203 sumptions for the growth functions $f_0(S_0, S_1)$ and $f_1(S_1)$:

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

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

206 **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 $S_1 > 0$, $\frac{df_1}{dS_1}(S_1) > 0$.

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

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

$$217 \quad s_0 = Y_2 S_0, \quad x_0 = \frac{Y_2}{Y_0} X_0, \quad s_1 = S_1, \quad x_1 = \frac{1}{Y_1} X_1, \quad (8)$$

218 We obtain the following system

$$219 \quad \begin{cases} \frac{ds_0}{dt} = D(s_0^{in} - s_0) - \mu_0(s_0, s_1)x_0 \\ \frac{dx_0}{dt} = -Dx_0 + \mu_0(s_0, s_1)x_0 - a_0x_0 \\ \frac{ds_1}{dt} = -Ds_1 + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1 \\ \frac{dx_1}{dt} = -Dx_1 + \mu_1(s_1)x_1 - a_1x_1 \end{cases} \quad (9)$$

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

$$221 \quad \mu_0(s_0, s_1) = Y_0 f_0\left(\frac{1}{Y_2} s_0, s_1\right) \quad \text{and} \quad \mu_1(s_2) = Y_1 f_1(s_2) \quad (10)$$

222 The functions μ_0 and μ_1 are general functions with their own properties. Since
 223 the functions f_0 and f_1 satisfy hypotheses **A1–A4**, it follows from (10) that
 224 functions μ_0 and μ_1 satisfy:

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

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

227 **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 $s_1 > 0$, $\frac{d\mu_1}{ds_1}(s_1) > 0$.

229 It should be noticed that (9) was studied in [10, 19] in the case where main-
 230 tenance effects are not taken into account, i.e. $a_0 = a_1 = 0$. We can easily
 231 prove that that for every non-negative initial condition, the solution of (9) has
 232 non-negative components and is positively bounded and thus is defined for every
 233 positive t .

234 4. Steady-state and stability analysis

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

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

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

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

$$-Dx_1 + \mu_1(s_1)x_1 - a_1x_1 = 0 \quad (14)$$

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

$$239 \quad x_0 = 0 \quad \text{or} \quad \mu_0(s_0, s_1) = D + a_0 \quad (15)$$

240 and from equation (14) we deduce that:

$$241 \quad x_1 = 0 \quad \text{or} \quad \mu_1(s_1) = D + a_1 \quad (16)$$

242 The case $x_0 = 0$ and $x_1 > 0$ is excluded. Indeed, as a consequence of (16), we
 243 have $\mu_1(s_1) = D + a_1$ and, as a consequence of (13), we have $Ds_1 + (D + a_1)x_1 =$
 244 0 , which is impossible since $s_1 \geq 0$ and $x_1 > 0$. Therefore, three cases must be
 245 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.

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

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

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

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

$$252 \quad s_1 = M_1(y) \iff y = \mu_1(s_1) \quad (17)$$

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

$$255 \quad s_0 = M_0(y, s_1) \iff y = \mu_0(s_0, s_1) \quad (18)$$

256 The inverse functions $s_1 = M_1(y)$ and $s_0 = M_0(y, s_1)$ can be calculated explic-
257 itly in the case of the Monod growth functions (23) considered in Section 6, see
258 formulas (25,(26)). We define the functions:

$$259 \quad \begin{aligned} F_0(D) &= M_0(D + a_0, 0) \\ F_1(D) &= M_1(D + a_1) + M_0(D + a_0, M_1(D + a_1)) \end{aligned} \quad (19)$$

260 Notice that $F_1(D) > F_0(D)$ for all $D \geq 0$, as long as they are both defined with
261 the exception $F_1(0) = F_0(0)$, which holds if and only if $a_0 = a_1 = 0$. Now, we
262 can describe the steady-states of (9).

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

$$265 \quad \bullet \text{ SS0} = (s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0)$$

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

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

273 The proof is given in the Appendix.

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

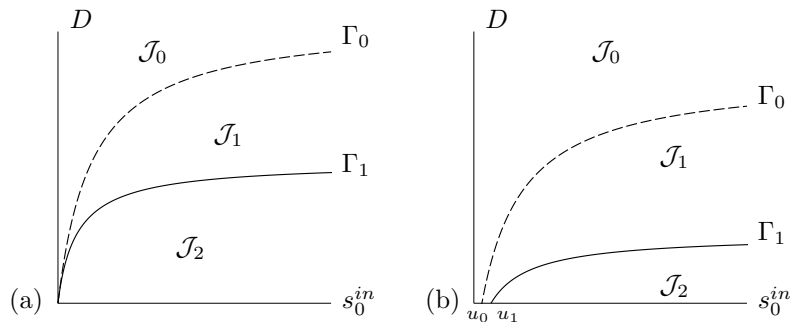


Figure 1: Operating diagram without (a) and with (b) maintenance effects. The values u_0 and u_1 are defined by (20)

282 5. Operating diagram

283 The operating diagram shows how the system behaves when we vary the two
 284 control parameters S_0^{in} and D . Let $F_0(D)$ and $F_1(D)$ be the functions defined

Condition	Region	SS0	SS1	SS2
$s_0^{in} < F_0(D)$	$(s_0^{in}, D) \in \mathcal{J}_0$	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.

285 by (19). The curve Γ_0 of equation $s_0^{in} = F_0(D)$ is the border which makes SS0
286 unstable and at the same time SS1 exists (the dashed curve in Fig. 1). The
287 curve Γ_1 of equation $s_0^{in} = F_1(D)$ is the border which makes SS1 unstable and
288 at the same time SS2 exists (the solid curve in Fig. 1).

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

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

$$294 \quad u_0 = F_0(0) = M_0(a_0, 0), \quad u_1 = F_1(0) = M_1(a_1) + M_0(a_0, M_1(a_1)), \quad (20)$$

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’

296 The model considered in [31] involves a two-tiered microbial ‘food chain’ with
297 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

$$\begin{cases}
 \frac{dS_{pro}}{dt} = D(S_{pro,in} - S_{pro}) - f_0(S_{pro}, S_{H_2}) X_{pro} \\
 \frac{dX_{pro}}{dt} = -DX_{pro} + Y_{pro}f_0(S_{pro}, S_{H_2}) X_{pro} - k_{dec,pro}X_{pro} \\
 \frac{dS_{H_2}}{dt} = -DS_{H_2} + \beta(1 - Y_{pro})f_0(S_{pro}, S_{H_2}) X_{pro} - f_1(S_{H_2}) X_{H_2} \\
 \frac{dX_{H_2}}{dt} = -DX_{H_2} + Y_{H_2}f_1(S_{H_2}) X_{H_2} - k_{dec,H_2}X_{H_2}
 \end{cases} \quad (21)$$

301 where S_{pro} and X_{pro} are propionate substrate and biomass concentrations; S_{H_2}
 302 and X_{H_2} are those for hydrogen; Y_{pro} and Y_{H_2} are yield coefficients and $\beta = 0.43$
 303 represents the part which goes to hydrogen substrate. The rest ($1 - \beta = 0.57$)
 304 goes to acetate which is left out in the model. Both growth functions take
 305 Monod form with an hydrogen inhibition for the first one

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

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

318 The basic results of the analysis of [31] are: for any pair of values of operating
 319 parameters, at most one steady-state is stable. When one of the decay terms is
 320 not taken into account, i.e. $k_{dec,pro} = 0$ or $k_{dec,H_2} = 0$ in (21), there is always
 321 one and only one steady-state which is stable and SS2 is stable as long as it
 322 exists. When both decay effects are present, i.e. $k_{dec,pro} > 0$ and $k_{dec,H_2} > 0$

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

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

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

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

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

$$333 \quad \mu_0(s_0, s_1) = \frac{m_0 s_0}{K_0 + s_0} \frac{1}{1 + s_1/K_i}, \quad \mu_1(s_1) = \frac{m_1 s_1}{K_1 + s_1} \quad (23)$$

334 where

$$335 \quad \begin{aligned} m_0 &= Y_0 k_{m,pro}, & K_0 &= Y_2 K_{s,pro}, & K_i &= K_{I,H_2} \\ m_1 &= Y_1 k_{m,H_2}, & K_1 &= K_{s,H_2} \end{aligned} \quad (24)$$

336 Let us describe our results in the particular case (9,23). Notice that the growth
 337 functions (23) satisfy Assumptions **H1**–**H4**, so that Proposition 1 holds. In this
 338 case the inverse functions $M_1(y)$ and $y \mapsto M_0(y, s_1)$ of the functions $\mu_1(s_1)$ and
 339 $s_0 \mapsto \mu_0(s_0, s_1)$ can be calculated explicitly: we have

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

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

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

343 by

$$\begin{aligned}
 F_0(D) &= \frac{K_0(D + a_0)}{m_1 - D - a_0} \\
 F_1(D) &= \frac{K_1(D + a_1)}{m_1 - D - a_1} + \frac{K_0(D + a_0)}{\frac{m_0}{1 + \frac{K_1(D+a_1)}{(m_1-D-a_1)K_i}} - D - a_0}
 \end{aligned} \tag{27}$$

345 Notice that F_0 is defined on $[0, m_1 - a_0)$ and F_1 is defined on $[0, D^+)$ with $D^+ <$
 346 $m_1 - a_0$. On the other hand, the solution s_0 of equation $\mu_0(s_0, s_0^{in} - s_0) = D + a_0$,
 347 which is used in SS1, is simply the positive solution of the quadratic equation:

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

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

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

- 352 • $SS0 = (s_0 = s_0^{in}, x_0 = 0, s_1 = 0, x_1 = 0)$
 353 *It always exists. It is stable if and only if $s_0^{in} < F_0(D)$.*
- 354 • $SS1 = (s_0, x_0 = \frac{D}{D+a_0} (s_0^{in} - s_0), s_1 = s_0^{in} - s_0, x_1 = 0)$
 355 *where s_0 is the positive solution of the quadratic equation (28). It exists*
 356 *if and only if $s_0^{in} > F_0(D)$. If it exists then it is stable if and only if*
 357 *$s_0^{in} < F_1(D)$.*
- $SS2 = (s_0, x_0 = \frac{D}{D+a_0} (s_0^{in} - s_0), s_1, x_1 = \frac{D}{D+a_1} (s_0^{in} - s_0 - s_1))$

where

$$s_1 = \frac{K_1(D + a_1)}{m_1 - D - a_1}, \quad s_0 = \frac{K_0(D + a_0)}{1 + \frac{s_1}{K_i} - D - a_0}$$

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

359 As a consequence of this result we obtain the results of [31], where the stabil-
 360 ity of SS2 was proved only for a particular set of parameter values. To make the
 361 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	$kg\ COD/m^3$	0.124
m_1	d^{-1}	2.10
K_1	$kg\ COD/m^3$	$2.5 \cdot 10^{-5}$
K_i	$kg\ COD/m^3$	$3.5 \cdot 10^{-6}$
a_0	d^{-1}	0.02
a_1	d^{-1}	0.02

Table 3: Nominal parameters values.

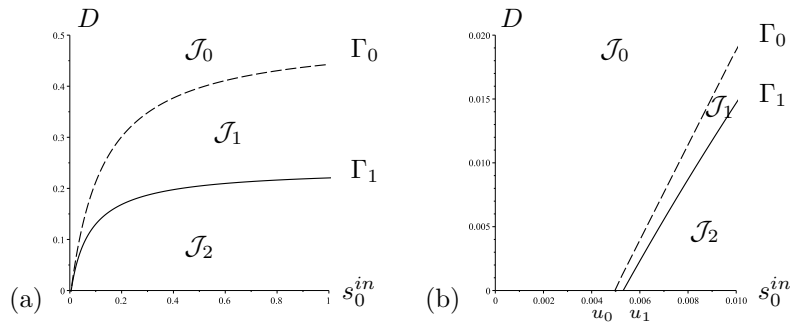


Figure 2: Operating diagram of the model (9)-(23). (a) The model was parametrised with the ADM1 consensus values listed in Table 3. (b) A magnification showing the values $u_0 = 4.96 \cdot 10^{-3}$, $u_1 = 5.31 \cdot 10^{-3}$ defined by (20).

368 For the numerical simulations we will use the nominal values of Table 3
 369 obtained from Table 1 of [31] by using the formulas (24) and $a_0 = k_{dec,pro}$,
 370 $a_1 = k_{dec,H_2}$. For these values of the parameters, the values u_0 and u_1 are very
 371 small, see Fig. 2. Notice that the scaling on the two coordinates in Fig. 2 are

372 different from those of Fig. 2 of [31], since these authors used another rescaling.

373 **7. Discussion**

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

388 In [31], the authors point out that introducing decay or maintenance in
389 the classical predator-prey models results in instability and chaos [13]. For
390 more details on food-chains in the chemostat the reader may consult [6, 24, 25].
391 Therefore, they observed that, in spite of the fundamental differences between
392 their ‘food chain’ and the classical predator-prey models, the same intrinsic
393 effect of maintenance on the stability of the food chain is observed [see 31,
394 Section 7]: When maintenance is included in its description, the two-tiered
395 generalized ‘food chain’ is not necessarily stable in theory. The results obtained
396 in the present paper indicate that the two-tiered generalized ‘food chain’ is
397 always stable, so that it is fundamentally different from the classical predator-
398 prey model.

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

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

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

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

432 ties while maintenance was not considered. In particular, the modified model
 433 exhibits multiplicity of positive steady-states. However, it should be stressed
 434 that these results were very general: whether this instability occurs for realistic
 435 environmental conditions or not is under investigation.

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

441 **Appendix A. Stability analysis**

442 We give the proof of Prop. 1. A steady-state (s_0, x_0, s_1, x_1) of (9) is a solution
 443 of the set of algebraic equations (11-14). The local stability of each steady-state
 444 depends on the sign of the real parts of the eigenvalues of the corresponding
 445 Jacobian matrix for the system (9). This is the matrix of the partial derivatives
 446 of the right hand side with respect to the state variables evaluated at the given
 447 steady-state (s_0, x_0, s_1, x_1) , that is:

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

where

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

449 The eigenvalues of J are the roots of its characteristic polynomial $\det(J - \lambda I)$.
 450 Notice that we have used the opposite sign for the partial derivative $F =$
 451 $-\frac{\partial \mu_0}{\partial s_1}(s_0, s_1)$, so that all constants involved in the computations become posi-
 452 tive, 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 $\lambda_3 = \lambda_4 = -D$.

455 For being stable we need $\lambda_1 < 0$. Therefore SS0 is unstable if and only if

456
$$\mu_0(s_0^{in}, 0) > D + a_0 \tag{B.1}$$

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)

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

459 Hence $x_0 = \frac{D}{D+a_0}(s_0^{in} - s_0)$ and $D(s_0^{in} - s_0) = Ds_1$, so that $s_0 + s_1 = s_0^{in}$.

460 Therefore s_0 is a solution of equation

461
$$\mu_0(s_0, s_0^{in} - s_0) = D + a_0 \tag{C.1}$$

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:

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

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 $\lambda_3 + \lambda_4 = -[D + (E + F)x_0] < 0$,

463 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

$$465 \quad \mu_1(s_0^{in} - s_0) < D + a_1, \text{ where } s_0 \text{ is the solution of (C.1)} \quad (C.2)$$

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

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

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

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

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

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

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

$$470 \quad \mu_0(s_0, M_1(D + a_1)) = D + a_0 \tag{D.1}$$

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

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

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

$$\begin{aligned} f_1 &= Gx_1 + (E + F)x_0 + 2D \\ f_2 &= EGx_0x_1 + (2D + a_0)(E + F)x_0 + (2D + a_1)Gx_1 + D^2 \\ f_3 &= (2D + a_0 + a_1)EGx_0x_1 + D(D + a_0)(E + F)x_0 + D(D + a_1)Gx_1 \\ f_4 &= (D + a_0)(D + a_1)EGx_0x_1 \end{aligned}$$

471 Hence

$$472 \quad f_i > 0 \text{ for } i = 1 \dots 4 \tag{D.2}$$

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

$$f_1f_2 - f_3 = 2D^3 + \alpha_2D^2 + \alpha_1D + \alpha_0$$

where

$$\begin{aligned} \alpha_2 &= 4(Hx_0 + Gx_1) \\ \alpha_1 &= 2(Hx_0 + Gx_1)^2 + a_0Hx_0 + a_1Gx_1 \\ \alpha_0 &= EG(Hx_0 + Gx_1)x_0x_1 + a_0H^2x_0^2 + (a_0 + a_1)FGx_0x_1 + a_1G^2x_1^2 \end{aligned}$$

473 Thus

$$474 \quad f_1f_2 - f_3 > 0 \tag{D.3}$$

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

$$\begin{aligned} \beta_5 &= 2(Hx_0 + Gx_1) \\ \beta_4 &= 4(Hx_0 + Gx_1)^2 + 2a_0Hx_0 + 2a_1Gx_1 \\ 475 \quad \beta_3 &= 2(Hx_0 + Gx_1)^3 + 4EG(Hx_0 + Gx_1)x_0x_1 \\ &\quad + 5a_0H^2x_0^2 + (a_0 + a_1)(3E + 5F)Gx_0x_1 + 5a_1G^2x_1^2 \end{aligned}$$

476

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

477

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

478

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

479 Thus

$$480 \quad f_1f_2f_3 - f_1^2f_4 - f_3^2 > 0 \quad (\text{D.4})$$

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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