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Maintenance affects the stability of a two-tiered microbial ‘food chain’?

Aiping Xu^{a,1,*}, Jan Dolfing^b, Thomas P. Curtis^b, Gary Montague^a, Elaine Martin^a^a*School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle upon Tyne NE1 7RU, UK*^b*School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK*

Abstract

Microbial ‘food chains’ are fundamentally different from canonical food chains in the sense that the waste products of the organisms on one trophic level are consumed by organisms of the next trophic level rather than the organisms themselves. In the present paper we introduce a generalised model of a two-tiered microbial ‘food chain’ with feedback inhibition, after applying an appropriate dimensionless transformation, and investigate its stability analytically. We then parameterised the model with consensus values for syntrophic propionate degradation compiled by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes. Consumption of energy for all processes other than growth is called maintenance. In the absence of maintenance and decay the microbial ‘food chain’ is intrinsically stable, but when decay is included in the description this is not necessarily the case. We point out that this is in analogy to canonical food chains where introduction of maintenance in the description of a stable (equilibrium or limit cycle) predator-prey system generates chaos.

Keywords: Anaerobic wastewater treatment, dimensionless transformation, stability, maintenance

1. Introduction

Anaerobic digestion processes are increasingly applied as a waste treatment technology (Van Lier et al., 2001, 2008; Ramirez et al., 2009). One of its advantages is that the process requires no energy input for oxygenation but rather produces energy in the form of methane. Methanogenic degradation of organic material is a sequential process (Dolfing, 1988; Schink, 1997). A series of micro-organisms is involved in the various conversion steps of these compounds into the ultimate products of methane and carbon dioxide. Typically, the product of one conversion step is the substrate for the next organism in the chain; each organism lives off the waste product(s) of its predecessor. This paradigm shows some resemblance to the classical concept of a food chain, the difference being that it is not the organisms themselves but rather their products that serve as a food source for the organisms at the next trophic level (Dolfing and Prins, 1996). Surprisingly little is known about the stability of these ‘food chains’ (Shen et al., 2007). We therefore seek to investigate the stability of these microbial ‘food chains’ at

*Corresponding author. Tel: +44 24 7688 8599

Email addresses: xuaiping@yahoo.com (Aiping Xu), jan.dolfing@ncl.ac.uk (Jan Dolfing), tom.curtis@ncl.ac.uk (Thomas P. Curtis), gary.montague@ncl.ac.uk (Gary Montague), e.b.martin@ncl.ac.uk (Elaine Martin)

¹Present address: SIGMA, Faculty of Engineering and Computing, Coventry University, CV1 5FB, UK

a fundamental level and contrast the findings to what is known about the stability of canonical food chains. To this end we constructed a generalised model of a two-tiered microbial food chain. The model is essentially a stripped down version of anaerobic digestion model No. 1 (ADM1), the consensus model supported by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes, 2002), and includes options for decay, which is the consumption of energy for all processes other than growth, and feedback inhibition, a salient characteristic of metabolic interactions in methanogenic microbial communities, where hydrogen and acetate both play this role, although with vastly different kinetic characteristics. The model is made dimensionless in a way that allows us to investigate decay effects without running into scale imbalance problems, and analysed for bifurcations. According to the Routh-Hurwitz criteria the non-trivial steady state is possibly unstable when decay is included. However, simulation results with ADM1 consensus values indicate that the possibly unstable state is always stable as long as it is meaningful, that is, all its state values are non-negative.

2. The model

Anaerobic digestion has been the subject of considerable research effort, not least in the area of modelling (Mosey, 1983; Siegrist et al., 1993). The complexity of the process calls for complex models, or so it seems. The current ADM1 consensus model (IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes, 2002) has a staggering 32 dynamic state variables and successfully captures the necessary dynamics of the process. However, this model is far too complex to permit mathematical analysis of its nonlinear dynamics (Shen et al., 2007). In order to make such studies possible, we have therefore reduced the model to its very backbone, a two-tiered microbial ‘food chain’ with feedback inhibition, which encapsulates the essence of methanogenic degradation processes, see Figure 1. Degradation of propionate is a case in point. In methanogenic ecosystems syntrophic propionate degrading bacteria convert propionate into hydrogen and acetate, which are then converted into methane by two other trophic groups, the hydrogenotrophic and the acetoclastic methanogens (Koch et al., 1983; Schink, 1997). For thermodynamic reasons propionate degradation is extremely sensitive to accumulation of hydrogen. Thus in methanogenic ecosystems propionate degradation is only sustainable in the presence of hydrogenotrophic organisms (typically methanogens) as hydrogen scavengers (Dolfing, 1988). Syntrophic interactions define the methanogenic degradation of a wide variety of compounds, including short- and long-chain fatty acids, amino acids and aromatics (Schink, 1997). Thus our modelling efforts will be undertaken with propionate as a model substrate. To be able to execute a thoroughly mathematical analysis we study propionate degradation with hydrogen production while ignoring acetate production. However, the dimensionless transformations introduced later can be easily extended to the general case. Using the notation also used in ADM1 the model for a two-tiered ‘food-chain’ with feedback inhibition can be written as

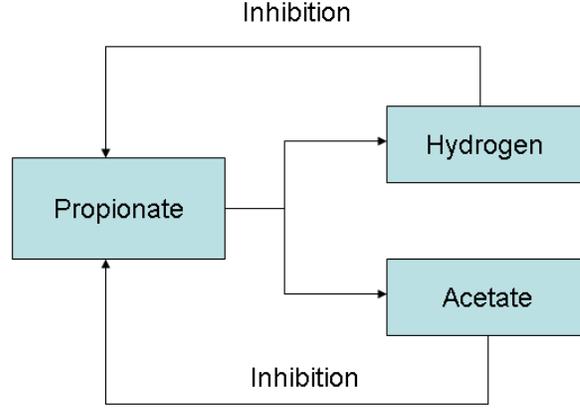


Figure 1: Propionate degradation with hydrogen and acetate inhibitions

$$\begin{cases} \frac{dS_{pro}}{dt} = D(S_{pro,in} - S_{pro}) - \frac{k_{m,pro}S_{pro}}{K_{s,pro} + S_{pro}} X_{pro} I_2 \\ \frac{dX_{pro}}{dt} = -DX_{pro} + Y_{pro} \frac{k_{m,pro}S_{pro}}{K_{s,pro} + S_{pro}} X_{pro} I_2 - k_{dec,pro} X_{pro} \\ \frac{dS_{H2}}{dt} = -DS_{H2} + 0.43(1 - Y_{pro}) \frac{k_{m,pro}S_{pro}}{K_{s,pro} + S_{pro}} X_{pro} I_2 - \frac{k_{m,H2}S_{H2}}{K_{s,H2} + S_{H2}} X_{H2} \\ \frac{dX_{H2}}{dt} = -DX_{H2} + Y_{H2} \frac{k_{m,H2}S_{H2}}{K_{s,H2} + S_{H2}} X_{H2} - k_{dec,H2} X_{H2} \end{cases} \quad (1)$$

where S_{pro} and X_{pro} are propionate substrate and biomass concentrations; S_{H2} and X_{H2} are those for hydrogen. 0.43 represents the part which goes to hydrogen substrate and the rest ($1 - 0.43 = 0.57$) goes to acetate which is left out in our model. Both growth functions take Monod form and the hydrogen inhibition function takes the non-competitive one: $I_2 = 1/(1 + S_{H2}/K_{I,H2})$. Other inhibition functions can be easily incorporated in this model. Here apart from the two environmentally controllable parameters: the inflowing propionate concentration $S_{pro,in}$ and the dilution rate D , the others all have biological meanings, with suggested values for mesophilic high rate (nom 35°C) taken from IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (2002), see Table 1.

We should pay special attention to the significantly different values of the half-saturation rates of hydrogen $K_{s,H2}$ and of propionate $K_{s,pro}$, which decide the scales of their corresponding concentrations in the chemostat.

Our aim is to study the stability of the model (1) while varying the two control parameters: the inflowing propionate concentration $S_{pro,in}$ and the dilution rate D , or more accurately to obtain the bifurcation diagram of (1). Too many biologically meaningful parameters hamper in our ability to identify factors that govern the stability of the model. Therefore, our first step is to find a suitable dimensionless transformation.

Parameters	Nominal values	Units
$k_{m,pro}$	13	COD/COD/d
$K_{s,pro}$	0.3	kgCOD/m ³
Y_{pro}	0.04	COD/COD
$k_{dec,pro}$	0.02	1/d
$k_{m,H2}$	35	COD/COD/d
$K_{s,H2}$	2.5×10^{-5}	kgCOD/m ³
Y_{H2}	0.06	COD/COD
$k_{dec,H2}$	0.02	1/d
$K_{I,H2}$	3.5×10^{-6}	kgCOD/m ³

Table 1: Parameters for a model of a two-tiered microbial ‘food chain’ with feedback inhibition, consisting of a propionate degrader and a hydrogenotrophic methanogen. The parameters are consensus values listed by IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (2002) for high rate mesophilic systems.

3. Dimensionless transformations

As pointed out in Gurney and Nisbet (1998), the behaviour of a natural system, where most variables and parameters have units, cannot be affected by the units in which we choose to measure the quantities we use to describe it. By using dimensionless transformation, we can reduce the number of parameters determining the dynamics, saving effort on numerical and analytic investigations. However, there is no unique dimensionless form for the equations. In considering the characteristics of the ADM1 model, which is ‘stiff’ due to the scale imbalance of different substrate concentrations (low hydrogen concentration), we introduce here the following dimensionless quantities, adapted from those described in Baltzis and Fredrickson (1984) but different from those commonly used for food chain models (Kot et al., 1992): all concentrations were rescaled somehow by the inflowing substrate concentration.

$$\tau \equiv k_{m,pro} Y_{pro}; \quad \alpha \equiv \frac{D}{k_{m,pro} Y_{pro}}; \quad u_f \equiv \frac{S_{pro,in}}{K_{s,pro}}$$

and

$$s_0 \equiv \frac{S_{pro}}{K_{s,pro}}; \quad x_0 \equiv \frac{X_{pro}}{K_{s,pro} Y_{pro}}$$

and

$$s_1 \equiv \frac{S_{H2}}{K_{s,H2}}; \quad x_1 \equiv \frac{X_{H2}}{K_{s,H2} Y_{H2}}$$

and

$$\phi \equiv \frac{k_{m,H2} Y_{H2}}{k_{m,pro} Y_{pro}}; \quad \omega \equiv \frac{K_{s,pro} \times 0.43(1 - Y_{pro})}{K_{s,H2}}$$

and

$$A \equiv \frac{k_{dec,pro}}{k_{m,pro}Y_{pro}}; \quad B \equiv \frac{k_{dec,H2}}{k_{m,pro}Y_{pro}}.$$

As a result we have the following dimensionless system:

$$\begin{aligned} \frac{dx_0}{d\tau} &= -\alpha x_0 + \mu_0 x_0 - Ax_0 \\ \frac{dx_1}{d\tau} &= -\alpha x_1 + \mu_1 x_1 - Bx_1 \\ \frac{ds_0}{d\tau} &= \alpha(u_f - s_0) - \mu_0 x_0 \\ \frac{ds_1}{d\tau} &= -\alpha s_1 + \omega \mu_0 x_0 - \mu_1 x_1 \end{aligned} \quad (2)$$

where

$$\mu_0 = \frac{s_0}{1 + s_0} I_2; \quad \mu_1 = \frac{\phi s_1}{1 + s_1}$$

$$\begin{aligned} I_2 &= \frac{1}{1 + S_{H2}/K_{I,H2}} \\ &= \frac{1}{1 + K_{s,H2} \times s_1/K_{I,H2}} \\ &= \frac{1}{1 + s_1/K_I} \end{aligned}$$

with $K_I \equiv K_{I,H2}/K_{s,H2}$.

Utilising this dimensionless transformation we have actually given the two dimensionless growth functions (propionate and hydrogen equivalents) the same half-saturation rates. Thus we have addressed the scale imbalance problem and at the same time introduced a generalised model, which describes the typical ADM1 framework with inhibition feedback and covers a wide range of microbial models.

Regarding to the case study of our two-tiered propionate degrading ‘food chain’, the parameters of the transformed model are:

$$\phi = 4.0385, \quad \omega = 4953.6, \quad A = B = 0.0385, \quad K_I = 0.14.$$

From now on we study the stability of the generalised model (2).

The steady state equations predict the following possible steady states (SS) for the system if categorised by the two microbial populations:

SS1: $x_0 = 0, x_1 = 0$: trivial solution where both populations are washed out;

SS2: $x_0 \neq 0, x_1 = 0$: hydrogen population is washed out while the host survives;

SS3: $x_0 \neq 0, x_1 \neq 0$: both populations survive.

A steady state is called meaningful if and only if all the concentrations are non-negative and $s_0 \leq u_f$. The local stability of each steady state will be tested by linearisation around the steady state values of the variables.

For a system of autonomous ordinary differential equations (ODEs), such as Eq. (2), the stability of its steady states depends on the signs of the real parts of the eigenvalues of the corresponding Jacobian matrix (Amundson, 1966). For any n -dimensional ODEs system of the form

$$\begin{cases} \frac{dx_1}{dt} = f_1(x_1, x_2, \dots, x_n) \\ \frac{dx_2}{dt} = f_2(x_1, x_2, \dots, x_n) \\ \vdots \\ \frac{dx_n}{dt} = f_n(x_1, x_2, \dots, x_n) \end{cases}$$

its Jacobian matrix is the matrix of the partial derivatives of the right hand side with respect to the state variables, that is

$$J = \left(\frac{\partial f_i}{\partial x_j} \right) = \begin{pmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \dots & \frac{\partial f_1}{\partial x_n} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} & \dots & \frac{\partial f_2}{\partial x_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial f_n}{\partial x_1} & \frac{\partial f_n}{\partial x_2} & \dots & \frac{\partial f_n}{\partial x_n} \end{pmatrix}$$

where all derivatives are evaluated at the steady state. A steady state is (asymptotically) stable if all eigenvalues have negative real parts. Otherwise, it is unstable. In the next two sections we will check the stability of all steady states one by one, with and without decay effects respectively, followed by numerical analysis of the ADM1 model as a case study.

4. Stability analysis without decay effects

The equations describing the generalised model, when decay effects are not taken into account (i.e. $A = B = 0$ in (2)), are written as the following:

$$\frac{dx_0}{d\tau} = -\alpha x_0 + \mu_0 x_0 \quad (3a)$$

$$\frac{dx_1}{d\tau} = -\alpha x_1 + \mu_1 x_1 \quad (3b)$$

$$\frac{ds_0}{d\tau} = \alpha(u_f - s_0) - \mu_0 x_0 \quad (3c)$$

$$\frac{ds_1}{d\tau} = -\alpha s_1 + \omega \mu_0 x_0 - \mu_1 x_1 \quad (3d)$$

By combining equations (3c) and (3a), we obtain

$$\frac{d}{dt}(s_0(t) + x_0(t)) = -\alpha(s_0(t) + x_0(t)) + \alpha u_f$$

As a result

$$s_0(t) + x_0(t) = u_f + (s_0(0) + x_0(0) - u_f)e^{-\alpha t}$$

where $s_0(0)$ and $x_0(0)$ are the initial concentrations of the rescaled propionate substrate and biomass. It follows that $s_0(t) + x_0(t) \rightarrow u_f$ as $t \rightarrow \infty$. We may, in other words, study the model's asymptotic behaviour along $s_0 + x_0 = u_f$.

With similar reasoning we can obtain the other constraint $s_1 - \omega x_0 + x_1 = 0$. Therefore, when asymptotic behaviour is concerned we can eliminate two state variables. Our system is thus equivalent to a two-dimensional one:

$$\begin{cases} \frac{dx_0}{d\tau} = -\alpha x_0 + \mu_0 x_0 \\ \frac{dx_1}{d\tau} = -\alpha x_1 + \mu_1 x_1 \end{cases}$$

where

$$\mu_0 = \frac{s_0}{1 + s_0} I_2; \quad I_2 = \frac{1}{1 + s_1/K_I}; \quad \mu_1 = \frac{\phi s_1}{1 + s_1}$$

with

$$s_0 = u_f - x_0; \quad s_1 = \omega x_0 - x_1.$$

Its Jacobian matrix is:

$$J = \begin{bmatrix} -\alpha + \mu_0 + (-E + \omega F)x_0 & -Fx_0 \\ \omega Gx_1 & -\alpha + \mu_1 - Gx_1 \end{bmatrix} \quad (4)$$

where

$$E = \frac{\partial \mu_0}{\partial s_0} > 0; \quad F = \frac{\partial \mu_0}{\partial s_1} < 0; \quad G = \frac{d\mu_1}{ds_1} > 0.$$

By inspecting the conditions under which each steady state is meaningful and stable, see Appendix A for detailed analysis, one can conclude that for any pair of values of the operating parameters, α and u_f , there is always one, and only one, steady state which is meaningful and stable.

5. Consideration of decay effects

Consumption of energy for all processes other than growth is called maintenance, since the processes involved effectively result in the maintenance of cell viability. In situations where microbial cells are located in a favourable environment, maintenance can often be neglected. In other situations, however, a significant portion of the energy-yielding substrate that could be used for growth is consumed for maintenance (Fredrickson and Tsuchiya, 1977). In the ADM1 model maintenance is taken into account as decay. In this section the generalised model (2) is studied, taking into consideration maintenance effects, which means $A > 0$ and $B > 0$. This system has the same possible steady states as those analysed in the previous section. Here we focus on the two positive terms A and B , to evaluate how the stability is affected by their simultaneous introduction.

The Jacobian matrix for this system is:

$$J = \begin{bmatrix} a_{11} & 0 & a_{13} & a_{14} \\ 0 & a_{22} & 0 & a_{24} \\ a_{31} & 0 & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{bmatrix} \quad (5)$$

where $a_{11} = -\alpha + \mu_0 - A$, $a_{13} = Ex_0$, $a_{14} = Fx_0$, $a_{22} = -\alpha + \mu_1 - B$, $a_{24} = Gx_1$, $a_{31} = -\mu_0$, $a_{33} = -\alpha - Ex_0$, $a_{34} = -Fx_0$, $a_{41} = \omega\mu_0$, $a_{42} = -\mu_1$, $a_{43} = \omega Ex_0$, $a_{44} = -\alpha + \omega Fx_0 - Gx_1$, with

$$E = \frac{\partial\mu_0}{\partial s_0} > 0; \quad F = \frac{\partial\mu_0}{\partial s_1} < 0; \quad G = \frac{d\mu_1}{ds_1}$$

having the same definitions as before.

A detailed study for this case has been performed, see Appendix B. Analytical conditions for each steady state to be meaningful have been derived and the characteristics of the eigenvalues have been studied for stability. Analytical expressions of the criteria for local stability have been developed and the Routh-Hurwitz criteria (Amundson, 1966) have been checked wherever necessary. The basic results of the analysis are: no steady state exhibits multiplicity; the steady states are mutually exclusive in the sense that for any pair of values for the operating parameters, α and u_f , at most one steady state can be meaningful and stable; Hopf bifurcation can possibly occur with the nontrivial steady state.

Remark 1 *The steady states depend on the assumption of a specific functional growth and inhibition. However, our method for the stability analysis is still effective for other functional forms as long as they keep the signs of their derivatives, both for the cases without and with decays, since any explicit form is not needed here.*

Remark 2 *As a special case when only one decay term is taken into consideration, the system can be reduced into a three-dimensional one. The corresponding Routh-Hurwitz criteria is checked and it is always satisfied so the nontrivial steady state is always stable as long as it is meaningful.*

6. Some simulation results

In consideration of the authors' specific interests in anaerobic digestion in wastewater treatment and the role of ADM1 in its modelling, we apply here its consensus parameters for the two-tiered propionate degrading methanogenic 'food-chain' to numerically check its stability, with maintenance neglected and included, respectively. We will present the operating diagrams, which are very generically useful in the case of the chemostat since when one decides to study a particular system in a chemostat all but two (the dimensionless dilution rate α and the dimensionless concentration of the substrate in the feed u_f) of the parameters which appear in the generalised model are fixed for they depend on the organisms and the substrates which have been picked. The operating parameters α and u_f can vary at our will. The operating diagram shows the regions where each steady state is stable. Therefore this diagram shows how the system behaves when we vary the two control parameters.

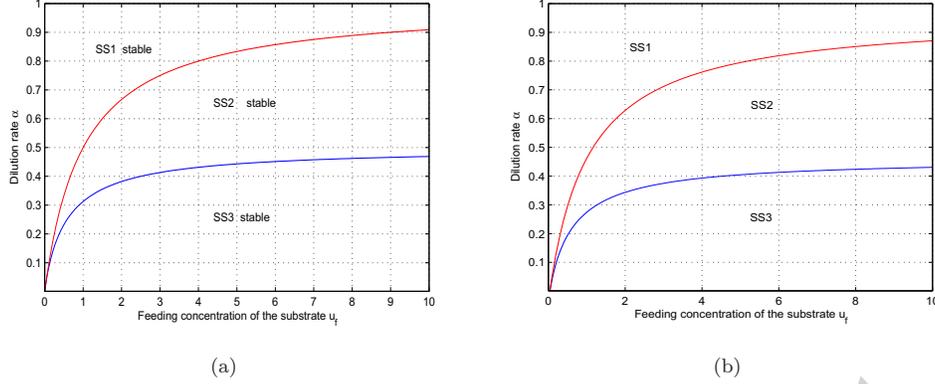


Figure 2: Bifurcation diagrams for a two-tiered microbial ‘food chain’ without (left) and with (right) maintenance effects. The model was parameterised with the ADM1 consensus values listed in Table 1

6.1. When maintenance effects are neglected

By applying the ADM1 parameters ($\phi = 4.0385$, $\omega = 4954$, $K_I = 0.14$) and neglecting the decay terms, which means $A = B = 0$, we obtain the bifurcation diagram Figure 2(a).

The dimensionless holding time is $\beta = 1/\alpha$. Recall the definition of the border which makes SS2 unstable and at the same time SS3 meaningful (the blue curve in Figure 2(a)) $F_1(s_1) = s_1^2 - (\omega(u_f + 1) + K_I(\beta - 1))s_1 + \omega K_I(u_f\beta - (u_f + 1)) = 0$ with $s_1 = 1/(\phi\beta - 1)$. Note that we can actually solve explicitly u_f as a function of β :

$$u_f = -\frac{1 - K_I\beta^2\phi - \omega K_I\phi^2\beta^2 - \omega\phi\beta + \omega - \omega K_I + K_I\beta + K_I\phi\beta - K_I + 2\omega K_I\phi\beta}{-\omega\phi\beta - 2\omega K_I\beta^2\phi + \omega K_I\beta + \omega K_I\beta^3\phi^2 - \omega K_I + \omega - \omega K_I\phi^2\beta^2 + 2\omega K_I\phi\beta}$$

6.2. When maintenance effects are included

With the ADM1 parameters, the two decay rates are equal, that is, $A = B = 0.0385$. By applying the ADM1 kinetics ($\phi = 4.0385$, $\omega = 4954$, $K_I = 0.14$ and $A = B = 0.0385$) we obtain the partition of the plane, where different steady states become meaningful, see Figure 2(b). As discussed before the only possibility of Hopf bifurcation occurs at the steady state SS3 when the two maintenance effects are included, thus we focus on it to see how to verify its stability, mathematically and numerically and what needs to be further addressed, when a specific case is considered.

1. Steady state SS3:

SS3 is the solution of the following algebraic equations:

$$\begin{cases} -\alpha x_0 + \mu_0 x_0 - A x_0 = 0 \\ -\alpha x_1 + \mu_1 x_1 - B x_1 = 0 \\ \alpha(u_f - s_0) - \mu_0 x_0 = 0 \\ -\alpha s_1 + \omega \mu_0 x_0 - \mu_1 x_1 = 0 \end{cases}$$

where

$$\mu_0 = \frac{s_0}{1+s_0}I_2; \quad I_2 = \frac{1}{1+s_1/K_I}; \quad \mu_1 = \frac{\phi s_1}{1+s_1}.$$

It is easy to get that $s_1 = (\alpha + B)/(\phi - (\alpha + B))$. If we denote $I_2 = 1/(1 + s_1/K_I)$ then $s_0 = (\alpha + A)/(I_2 - (\alpha + A))$, $x_0 = \alpha(u_f - s_0)/(\alpha + A)$ and $x_1 = (-\alpha s_1 + \omega\alpha(u_f - s_0))/(\alpha + B)$. The partition between SS2 and SS3 satisfies:

$$F_1(s_1) = s_1^2 - \left[\omega(u_f + 1) + K_I \left(\frac{1}{\alpha + A} - 1 \right) \right] s_1 + \omega K_I \left(\frac{u_f}{\alpha + A} - (u_f + 1) \right) = 0$$

where

$$s_1 = \frac{1}{\frac{\phi}{\alpha + A} - 1}$$

Moreover, at SS3,

$$\begin{aligned} E &= \frac{I_2}{(1+s_0)^2} = \frac{(I_2 - (\alpha + A))^2}{I_2} \\ F &= \frac{s_0}{1+s_0} \left(-\frac{I_2^2}{K_I} \right) = -\frac{(\alpha + A)I_2}{K_I} \\ G &= \frac{\phi}{(1+s_1)^2} = \frac{(\phi - (\alpha + B))^2}{\phi} \end{aligned}$$

2. Characteristic Polynomial at SS3

From (B.2), the characteristic polynomial at SS3 is

$$\lambda^4 + f_1\lambda^3 + f_2\lambda^2 + f_3\lambda + f_4 = 0$$

where

$$f_1 = Ex_0 - \omega Fx_0 + Gx_1 + 2\alpha$$

$$f_2 = -\omega AFx_0 + Ex_0Gx_1 + 2Ex_0\alpha + \alpha^2 + AEx_0 - 2\alpha\omega Fx_0 + 2\alpha Gx_1 + Bx_1G$$

$$f_3 = AEx_0\alpha - \omega AFx_0\alpha - \omega\alpha^2 Fx_0 + 2Gx_1Ex_0\alpha + Gx_1BEx_0 + AEx_0Gx_1 + Gx_1B\alpha + Gx_1\alpha^2 + Ex_0\alpha^2$$

$$f_4 = AGx_1BEx_0 + \alpha Gx_1BEx_0 + Gx_1Ex_0\alpha^2 + AGx_1Ex_0\alpha$$

As discussed above, all the polynomial coefficients are only functions of the two control parameters α and u_f .

3. Stability check

We further numerically check the stability of SS3. From the Routh-Hurwitz criteria ($f_1f_2f_3 > f_3^2 + f_1^2f_4$) we define a function $F = f_1f_2f_3 - (f_3^2 + f_1^2f_4)$, which depends only on the two control parameters α and u_f . See Figure 3 as its surface description. The numeric results show that SS3 is always stable as long as it is meaningful, despite the fact that it can be theoretically unstable, see Section 5, where the internal constraints of the variables are not taken into account when considering a generalised model.

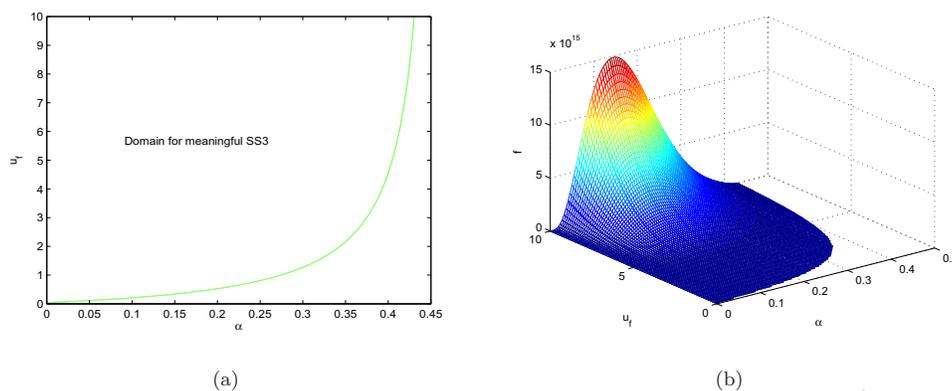


Figure 3: ADM1 stability check at SS3. (a) The part that SS3 is meaningful, duplicated from Figure 2(a); (b) The surface of function F for stability check at SS3.

7. Discussion

The stability of food chains and food webs has been the subject of numerous studies and it has been shown that many factors contribute in stabilising the system (Gross, 2004; Gross et al., 2006, 2009; May, 1974). Here we point out that maintenance appears to be a key factor when a two-tiered microbial 'food chain' is considered. Kot et al. (1992) have shown that the populations of the protozoan predator and bacterial prey in a chemostat with an constant inflowing substrate, can coexist on a limit cycle, while Kooi and Boer (2003) have numerically shown that similar structured model can generate chaos. The difference between the models used by the different groups is that, unlike Kooi and Boer (2003), Kot et al. (1992) do not include maintenance. In spite of the fundamental differences between our 'food chain' and the classical predator-prey discerned in macro ecology we observe the same intrinsic effect of maintenance on the stability of the food chain. In the absence of maintenance our two-tiered 'food chain' too is always stable, but when maintenance is included in its description our two-tiered generalised 'food chain' is not necessarily stable in theory. However, using the consensus parameters of ADM1 we have shown in numerical simulations that our model of the methanogenic two-tiered propionate-hydrogen food chain is always stable. Direct application of symbolic analysis programs, such as Maple or Mathematica, turned out fruitless. Stiefs et al. (2008) have proposed a more effective way to compute and visualise bifurcation surfaces, which enhance the qualitative understanding of a system and can help to quickly locate more complex bifurcation situations corresponding to bifurcations. Moreover, numerical analysis using programs like AUTO (Doedel et al., 1997) can hopefully help to deal with the problem proposed here like in Kooi et al. (1998). Both will serve as our tools to pinpoint the conditions under which our generalised system is prone to becoming unstable. For the operators of anaerobic wastewater treatment systems our results are good news and bad news. Good because we have shown that the syntrophic associations between propionate degraders and methanogens are inherently stable under realistic environmental conditions. The bad news though is that it is not a priori possible to generalise; there may well be other two-tiered

‘food chains’ that become unstable under environmentally realistic conditions, which are the authors’ current research interest.

Hess and Bernard (2008) pointed out that the excessive complexity of ADM1 makes any advanced mathematical analysis critical. The present analysis is based on a two-tiered model but it opens up the questions about the stability of longer microbial ‘food chains’ and more intricate ‘food webs’. Computational methods can be taken into consideration. For example Gross et al. (2005) have demonstrated that canonical long food chains are in general chaotic, after applying the steady state under consideration to normalise the ODE system.

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Appendix A. Stability analysis with maintenance neglected

SS1: $x_0 = 0, x_1 = 0$. As a consequence $s_0 = u_f$ and $s_1 = 0$. This steady state is always meaningful. Since $\mu_0 = u_f/(1 + u_f)$ and $\mu_1 = 0$, the Jacobian matrix (4) becomes

$$J = \begin{bmatrix} -\alpha + \frac{u_f}{1+u_f} & 0 \\ 0 & -\alpha \end{bmatrix}$$

Its eigenvalues are $\lambda_1 = -\alpha + u_f/(1 + u_f)$ and $\lambda_2 = -\alpha$. SS1 is a node since all eigenvalues are real; for being stable it must be $\lambda_1 < 0$. Therefore, SS1 is meaningful and stable if and only if $\alpha > u_f/(1 + u_f)$.

SS2: $x_0 \neq 0, x_1 = 0$. As a result $\mu_0 = s_0/(1 + s_0)/(1 + s_1/K_I) = \alpha$ and $s_1 = \omega x_0$. Note that $s_0 + x_0 = u_f$. Thus s_0 satisfies the following quadratic equation:

$$F_0(s_0) = s_0^2 + \left(\frac{K_I}{\omega} \left(\frac{1}{\alpha} - 1 \right) + (1 - u_f) \right) s_0 - \left(\frac{K_I}{\omega} + u_f \right) = 0 \quad (\text{A.1})$$

Due to the fact that $-(K_I/\omega + u_f) < 0$, there is only one unique positive real solution for the equation (A.1). To guarantee that SS2 is meaningful all that we need is $s_0 < u_f$, that is, $F_0(u_f) = K_I(u_f - \alpha(u_f + 1))/\alpha/\omega > 0$, which is equivalent to $\alpha < u_f/(1 + u_f)$. This implies that as soon as SS1 becomes unstable, SS2 becomes meaningful.

On the other hand, s_1 satisfies the following quadratic equation:

$$F_1(s_1) = s_1^2 - \left[\omega(u_f + 1) + K_I \left(\frac{1}{\alpha} - 1 \right) \right] s_1 + \omega K_I \left(\frac{u_f}{\alpha} - (u_f + 1) \right) = 0 \quad (\text{A.2})$$

As previous reasoning $\alpha < u_f/(1 + u_f)$, we have $\omega K_I(u_f/\alpha - (u_f + 1)) > 0$ and $\omega(u_f + 1) + K_I(1/\alpha - 1) > 0$. We further check its discriminant $\Delta = [\omega(u_f + 1) + K_I(1/\alpha - 1)]^2 - 4\omega K_I(u_f/\alpha - (u_f + 1)) = [\omega(u_f + 1) - K_I(1/\alpha - 1)]^2 + 4\omega K_I/\alpha > 0$. Consequently there are two real positive roots for the equation (A.2). Since $s_1 < \omega u_f$ and $F_1(\omega u_f) = -\omega^2 u_f - \omega K_I < 0$, only the smaller root is meaningful, which we denote as s_1^* .

The Jacobian matrix (4) becomes

$$J = \begin{bmatrix} (-E + \omega F)x_0 & -Fx_0 \\ 0 & -\alpha + \mu_1 \end{bmatrix}$$

Its eigenvalues are $\lambda_1 = (-E + \omega F)x_0 < 0$ and $\lambda_2 = -\alpha + \mu_1$. It is also a node and SS2 is stable if and only if $\alpha > \mu_1$, where $\mu_1 = \phi s_1/(1 + s_1)$.

$$\alpha > \mu_1 \iff s_1 < \frac{\alpha}{\phi - \alpha} = \frac{1}{\phi/\alpha - 1}$$

which implies $F_1(1/(\phi/\alpha - 1)) < 0$.

SS3: $x_0 \neq 0, x_1 \neq 0$. As a result $\mu_0 = s_0/(1 + s_0)/(1 + s_1/K_I) = \alpha$ and $\mu_1 = \phi s_1/(1 + s_1) = \alpha$, which means $s_1 = \alpha/(\phi - \alpha)$. If we denote $I_2 = 1/(1 + s_1/K_I)$ then $s_0 = \alpha/(I_2 - \alpha)$, $x_0 = u_f - s_0$ and $x_1 = -s_1 + \omega(u_f - s_0)$. So to guarantee that SS3 is meaningful we must have

$$s_1 > 0 \iff \alpha < \phi; \tag{A.3a}$$

$$s_0 > 0 \iff \alpha < I_2; \tag{A.3b}$$

$$x_0 > 0 \iff s_0 < u_f; \tag{A.3c}$$

$$x_1 > 0 \iff s_1 < \omega(u_f - s_0) \tag{A.3d}$$

First we consider (A.3a) and (A.3b):

$$\begin{aligned} \alpha < I_2 &= \frac{1}{1 + s_1/K_I} = \frac{K_I}{K_I + s_1} \\ \iff s_1 &< \frac{K_I(1 - \alpha)}{\alpha} = K_I \left(\frac{1}{\alpha} - 1 \right). \end{aligned}$$

Then from (A.3c):

$$\begin{aligned} s_0 &= \frac{\alpha}{I_2 - \alpha} = \frac{\alpha}{\frac{K_I}{K_I + s_1} - \alpha} < u_f \\ \iff s_1 &< K_I \left(\frac{u_f}{\alpha(u_f + 1)} - 1 \right). \end{aligned}$$

So $s_1 < K_I(u_f/\alpha/(u_f + 1) - 1)$. Moreover,

$$F_1 \left(K_I \left(\frac{u_f}{\alpha(u_f + 1)} - 1 \right) \right) = \frac{K_I^2(-u_f + \alpha(1 + u_f))}{\alpha^2(1 + u_f)^2} < 0$$

And from (A.3d):

$$\begin{aligned}
& s_1 < \omega(u_f - s_0) \\
\iff & s_1^2 - \left[\omega(u_f + 1) + K_I \left(\frac{1}{\alpha} - 1 \right) \right] s_1 + \omega K_I \left(\frac{u_f}{\alpha} - (u_f + 1) \right) > 0
\end{aligned}$$

Recall the definition of F_1 and $s_1 = 1/(\phi/\alpha - 1)$ we have $F_1(1/(\phi/\alpha - 1)) > 0$.

The Jacobian matrix (4) becomes

$$J = \begin{bmatrix} (-E + \omega F)x_0 & -Fx_0 \\ \omega Gx_1 & -Gx_1 \end{bmatrix}$$

Its eigenvalues are given as roots of the equation

$$\lambda^2 + [(E - \omega F)x_0 + Gx_1]\lambda + EGx_0x_1 = 0.$$

Since $E > 0$, $F < 0$ and $G > 0$, it is obvious that the two eigenvalues λ_1 and λ_2 satisfy $\lambda_1 + \lambda_2 < 0$ and $\lambda_1\lambda_2 > 0$. Moreover, the discriminant of the above equation is $\Delta = [(E - \omega F)x_0 + Gx_1]^2 - 4EGx_0x_1$ and it can be either positive or negative. Therefore, the two eigenvalues can either be real and negative or complex conjugate with negative real parts. Hence as long as it exists it is stable. It can never be $\lambda_1 + \lambda_2 = 0$, hence Hopf bifurcation cannot occur from SS3.

Appendix B. Stability analysis with maintenance included

SS1: $x_0 = 0$, $x_1 = 0$. As a result $s_0 = u_f$ and $s_1 = 0$. This steady state is always meaningful.

The Jacobian matrix (5) becomes

$$J = \begin{bmatrix} -\alpha + \mu_0 - A & 0 & 0 & 0 \\ 0 & -\alpha - B & 0 & 0 \\ -\frac{u_f}{1+u_f} & 0 & -\alpha & 0 \\ \frac{\omega u_f}{1+u_f} & 0 & 0 & -\alpha \end{bmatrix}$$

Its eigenvalues are $\lambda_1 = -\alpha + u_f/(1 + u_f) - A$, $\lambda_2 = -\alpha - B < 0$ and $\lambda_3 = \lambda_4 = -\alpha < 0$. SS1 is a node since all eigenvalues are real; for it being stable we need $\lambda_1 < 0$. Therefore, SS1 is meaningful and stable if and only if $\alpha > u_f/(1 + u_f) - A$.

SS2: $x_0 \neq 0$, $x_1 = 0$. Consequently, $\mu_0 = s_0/(1 + s_0)/(1 + s_1/K_I) = \alpha + A$, $(\alpha + A)x_0 = \alpha(u_f - s_0)$ and $\alpha s_1 = \omega(\alpha + A)x_0$. Just as the case without maintenance effects we have the constraint $s_1 = \omega(u_f - s_0)$. Thus s_0 satisfies the following quadratic equation:

$$F_0(s_0) = s_0^2 + \left(\frac{K_I}{\omega} \left(\frac{1}{\alpha + A} - 1 \right) + (1 - u_f) \right) s_0 - \left(\frac{K_I}{\omega} + u_f \right) = 0$$

As the same reasoning before, to guarantee that SS2 is meaningful all we need is $\alpha < u_f/(1 + u_f) - A$. Note that as long as SS1 becomes unstable, SS2 becomes meaningful.

And s_1 satisfies the following quadratic equation:

$$F_1(s_1) = s_1^2 - \left[\omega(u_f + 1) + K_I \left(\frac{1}{\alpha + A} - 1 \right) \right] s_1 + \omega K_I \left(\frac{u_f}{\alpha + A} - (u_f + 1) \right) = 0 \quad (\text{B.1})$$

As discussed in Appendix A there are two positive roots for the quadratic equation (B.1) but only the smaller one is meaningful.

The Jacobian matrix (5) becomes

$$J = \begin{bmatrix} 0 & 0 & Ex_0 & Fx_0 \\ 0 & -\alpha + \mu_1 - B & 0 & 0 \\ -(\alpha + A) & 0 & -\alpha - Ex_0 & -Fx_0 \\ \omega(\alpha + A) & -\mu_1 & \omega Ex_0 & -\alpha + \omega Fx_0 \end{bmatrix}$$

Its characteristic polynomial is

$$\begin{aligned} |\lambda I - J| &= (\lambda + \alpha - \mu_1 + B)(\lambda + \alpha)[\lambda^2 + (\alpha + (E - \omega F)x_0)\lambda + (\alpha + A)(E - \omega F)x_0] \\ &= 0 \end{aligned}$$

Its eigenvalues are $\lambda_1 = -\alpha + \mu_1 - B$, $\lambda_2 = -\alpha < 0$, and the other two λ_3 and λ_4 are given as roots of the following quadratic equation

$$\lambda^2 + [\alpha + (E - \omega F)x_0]\lambda + (\alpha + A)(E - \omega F)x_0 = 0.$$

So for being stable it must be $\lambda_1 < 0$, which implies $F_1(1/(\phi/(\alpha + B) - 1)) < 0$.

SS3: $x_0 \neq 0$; $x_1 \neq 0$. So $s_1 = (\alpha + B)/(\phi - (\alpha + B))$. If we denote $I_2 = 1/(1 + s_1/K_I)$ then $s_0 = (\alpha + A)/(I_2 - (\alpha + A))$, $x_0 = \alpha(u_f - s_0)/(\alpha + A)$ and $x_1 = (-\alpha s_1 + \omega\alpha(u_f - s_0))/(\alpha + B)$. As the case without maintenance effects, to guarantee SS3 is meaningful we must have $F_1(1/(\phi/(\alpha + B) - 1)) > 0$.

The Jacobian matrix (5) becomes

$$J = \begin{bmatrix} 0 & 0 & Ex_0 & Fx_0 \\ 0 & 0 & 0 & Gx_1 \\ -(\alpha + A) & 0 & -\alpha - Ex_0 & -Fx_0 \\ \omega(\alpha + A) & -(\alpha + B) & \omega Ex_0 & -\alpha + \omega Fx_0 - Gx_1 \end{bmatrix}$$

Its characteristic polynomial is

$$\begin{aligned} |\lambda I - J| &= (\lambda + \alpha)^2[\lambda^2 + [(E - \omega F)x_0 + Gx_1]\lambda + EGx_0x_1] + ABEGx_0x_1 \\ &\quad + Ax_0(\lambda + \alpha)[(E - \omega F)\lambda + EGx_1] + BGx_1(\lambda + \alpha)(\lambda + Ex_0) \\ &= 0 \end{aligned}$$

It explicitly indicates how the maintenance affects the system's long-term behaviour, which is never seen in any literature up to our knowledge.

It is obvious that the case, when only one decay ($A = 0$ and $B \neq 0$, or, $A \neq 0$ and $B = 0$) or no decay ($A = B = 0$), can be regarded as a special case of this one.

For the nontrivial case ($A, B > 0$), if we expand the above equation as

$$\lambda^4 + f_1\lambda^3 + f_2\lambda^2 + f_3\lambda + f_4 = 0 \quad (\text{B.2})$$

where

$$f_1 = Ex_0 - \omega Fx_0 + Gx_1 + 2\alpha$$

$$f_2 = -\omega AFx_0 + Ex_0Gx_1 + 2Ex_0\alpha + \alpha^2 + AEx_0 - 2\alpha\omega Fx_0 + 2\alpha Gx_1 + Bx_1G$$

$$f_3 = AEx_0\alpha - \omega AFx_0\alpha - \omega\alpha^2 Fx_0 + 2Gx_1Ex_0\alpha + Gx_1BEx_0 + AEx_0Gx_1 + Gx_1B\alpha + Gx_1\alpha^2 + Ex_0\alpha^2$$

$$f_4 = AGx_1BEx_0 + \alpha Gx_1BEx_0 + Gx_1Ex_0\alpha^2 + AGx_1Ex_0\alpha$$

Since $E > 0$, $F < 0$ and $G > 0$, all coefficients f_i , $i = 1, \dots, 4$ are positive. Therefore, (B.2) cannot have any positive roots. When the Routh-Hurwitz criteria ($f_1f_2f_3 > f_3^2 + f_1^2f_4$) are checked one can see that they are not necessary satisfied. Thus the roots of (B.2) can be real and negative, or pairs of complex conjugate numbers with either negative or positive real parts. The possibility of having (at least) one pair of complex eigenvalues with positive real parts implies that Hopf bifurcation can originate from SS3.

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