

## Substrate degradation by a mutualistic association of two species in the Chemostat

**Almut Burchard**

School of Mathematics, Georgia Institute of Technology, Atlanta, GA 30332, USA

Received 27 July 1991; received in revised form 5 January 1993

**Abstract.** We discuss a system of ordinary differential equations that can be used to model the *interspecies hydrogen transfer* common in anaerobic degradation of organic matter. The mutualistic character of the interaction is not modeled explicitly but emerges as a consequence of the kinetics of nutrient uptake. Using monotonicity assumptions on the reaction terms, we characterise the equilibria and their stability and demonstrate two-parameter bifurcation of periodic solutions near singularities of the Bogdanov-Takens type. We have persistence and extinction results in a wide range of parameter values. Finally, we give some conditions for equivalence and non-equivalence to a cooperative system and compare to related models.

**Key words:** Chemostat model – Interspecies hydrogen transfer – Cooperative system – Persistence – Bifurcation – Bogdanov-Takens singularities

### 1 Introduction

Methane is a common final product in the anaerobic degradation of organic matter [2, 18, 32]. Not much was known about the mechanism of methane production and the physiology of methane producing bacteria before Bryant et al. [4] proved that *methanobacterium omelianskii* is in fact an association of two species which are strongly mutualistic under natural conditions and happen to look alike under the microscope. The first species, the *S-organism*, degrades ethanol to acetate and hydrogen. The reaction has an unfavorable entropy balance unless hydrogen partial pressures are kept extremely low, so molecular hydrogen is not observed in any significant quantities in natural ecosystems. The second species, *methanobacterium bryantii*, produces methane and water from hydrogen and carbon dioxide; it uses acetate when offered but does not depend on it.

This interspecies hydrogen transfer plays an important role in anaerobic food chains [15, 22, 23]. Depending on environmental conditions, other hydrogen consumers like sulfate-reducing bacteria may compete with methanobacteria for the

hydrogen, replace them or coexist with them. For all these species, hydrogen is typically the limiting substrate. Hydrogen is produced by a variety of bacterial species degrading fatty acids [22], alcohols or aromates [23]. If no hydrogen consumers are present, they cannot oxidize their substrates completely and cannot grow well.

Another example of this type of interaction was observed between lactate producing and lactate consuming bacteria by Otto et al. [25]. Similar in structure is an association observed by Pollock [26] (see [24]), where a metabolic product that is toxic for the producer is consumed by a second species.

Many of these associations have been studied experimentally in continuous culture as well as in batch culture [e.g. 16, 29]. In their chapter of Wimpenny's book on laboratory model systems [11] Gottschal and Dijkhuizen argue that compared to more complex representations of ecosystems in the laboratory the simplicity of chemostat systems results in better experimental control and simpler analysis. Compared to batch cultures, on the other hand, the chemostat admits exponential growth in a steady state typically determined by one limiting substrate, as opposed to a succession of a growth period followed by a period of stagnation. Specifically for the interspecies hydrogen transfer, in the chemostat as well as in natural systems, hydrogen partial pressures remain low, whereas in the batch culture, accumulation occurs in a transient phase.

Spatial homogeneity is ensured by continuous mixing. This is a major restriction for the use of laboratory chemostats as models for natural ecosystems, but it also makes them easy to represent mathematically. Neglecting the time required for absorption, transportation and metabolic processes, and also neglecting variations of the interior state of the microorganisms, one naturally arrives at a system of ordinary differential equations.

Experimenting with two species exchanging methanol in the chemostat, Wilkinson et al. [30] also introduced model equations for such an interaction. Following ideas by Powell [27], Kreikenbohm and Bohl [17] constructed a model for the interspecies hydrogen transfer with a different expression for the kinetics of nutrient uptake.

It seemed to us an interesting model system to study mutualism in some detail. We are specifically interested in how the long-term behaviour is influenced by the experimentally controlled variables, influx substrate concentration and dilution rate, and by the kinetics of nutrient uptake. The characteristic growth rates as functions of the substrate concentrations can be determined in pure cultures. The state of the system at a given time is described by concentrations and population densities, which are measured during an experiment. No direct coupling terms are introduced. Mutualism emerges as a system property that may depend on the experimental parameters and also on the state of the system. The model equations are introduced in §2.

The Kreikenbohm-Bohl model will be generalized in two respects. First, considering the different expressions given by Kreikenbohm and Bohl and Wilkinson, the kinetics of nutrient uptake and growth will not be restricted to Michaelis-Menten type. In fact, for arbitrary monotonic uptake functions we completely analyse the dynamics. Using mass balances, we approximate the long-time behaviour with the solutions of a pair of differential equations (§§ 2, 6). These equations coincide with standard models for two mutualists as presented for example in May [21]. In particular, linear uptake functions lead to Volterra-type equations. They form a cooperative system, with coupling terms determined by the uptake functions and the experimen-

tal parameters. Using the work on cooperative systems by Hirsch [13] and Matano [20], we prove that the system always tends to equilibrium (Proposition 3 in §3); this equilibrium is determined in terms of parameters and initial values. We thus confirm the numerical results from Kreikenbohm and Bohl [17].

Second, to include the example observed by Pollock [24, 26], we allow for a death rate of the first species caused by toxic effects of its product. Existence and stability of the equilibria are discussed in §3 and summarised in a bifurcation diagram. Numerical solutions of initial value problems presented in [5] suggest that for monotonic uptake functions still all solutions approach equilibria; analytically, global results about long-time behaviour are difficult to obtain because even after reduction the system consists of three equations, so qualitative methods from the theory of planar flows cannot be applied. The main results are Proposition 1 on extinction and Proposition 2 on uniform persistence in §5.

In both cases, non-monotonic uptake functions lead to multiple stable equilibria. In §4 we use methods from two-parameter bifurcation theory to prove existence of periodic solutions for suitable right hand sides. We show that periodic solutions may be stable or unstable depending on the shape of the uptake functions.

In the final section §7 we summarise the results. We discuss the relation to direct-interaction models depending on monotonicity properties of the kinetics of nutrient uptake. We return to the question, when and in which sense the model describes a system of mutualists.

## 2 The model equations

We will describe the state of the system in the chemostat at any time by four quantities, the population densities  $u$  and  $v$  of the two species (“producer” and “consumer”), and the concentrations  $s$  and  $c$  of the substrate and metabolic product of the first species (“substrate” and “product”). In the case of *methanobacterium omelianskii*, the substrate is ethanol, the product, as always for the interspecies hydrogen transfer, is hydrogen, the producer is the *S-organism*, and the consumer *methanobacterium bryantii*. The first species is inhibited by its metabolic product. The second species uses this metabolic product as a substrate. We distinguish dilution rate,  $D$ , and the concentration of the limiting substrate in the influx,  $S^0$ , as experimental variables. We fix all the other parameters like temperature, light, or non-limiting substrates, incorporating their effects into the characteristic growth rates. This should give a useful description of the laboratory model if the chemostat is well stirred and all metabolic processes are fast compared to the doubling times of the bacteria and the reciprocal of the dilution rate.

We arrive at the system of equations

$$\begin{aligned} \dot{s} &= D(S^0 - s) - K_1 f(s, c)u \\ \dot{u} &= -Du + f(s, c)u - Ecu \\ \dot{c} &= -Dc + K_2 f(s, c)u - g(c)v \\ \dot{v} &= -Dv + K_3 g(c)v . \end{aligned}$$

The first equation describes how substrate flows into the vessel at concentration  $S^0$  with a dilution rate  $D$ . It is consumed and metabolised by the first species at a rate  $K_1 f(s, c)$  depending on the concentrations of both substrate and product. The third equation describes the conversion of substrate into product, the washout at rate  $D$

and the consumption of the product by the second species at rate  $g(c)$  depending on the product concentration. In the second and fourth equation, we assume that the growth of the population densities  $u$  and  $v$ , respectively, is instantaneous and proportional to substrate intake. There is no direct interaction between the species.  $Ec$  in the equation for the producer represents a death rate induced by toxic effects of the product.

We assume that the functions  $f$  and  $g$  are nonnegative and satisfy

$$\begin{aligned} f(s, c) &= \max \{ \tilde{f}(s, c), 0 \} \\ g(c) &= \max \{ \tilde{g}(c), 0 \} , \end{aligned}$$

where  $\tilde{f} : \mathbf{R}^2 \rightarrow \mathbf{R}$  and  $\tilde{g} : \mathbf{R} \rightarrow \mathbf{R}$  are continuously differentiable, and

$$\tilde{f}(0, 0) = 0, \quad \tilde{g}(0) = 0 .$$

Product inhibition is included by

$$\tilde{f}_c(s, c) := \frac{\partial}{\partial c} \tilde{f}(s, c) \leq 0 ,$$

and toxic product effects taking  $E > 0$ , introducing a death rate of the first species proportional to the product concentration  $c$ . If  $\tilde{f}_c - E < 0$ , then the intermediate product has some negative effect on the producer and the two species appear mutualistic. If  $f = f(s)$  and  $E = 0$ , the two species are merely commensal, and the system reduces to a food chain.  $K_1, K_2, K_3$  are characteristic conversion constants of substrate into biomass and metabolic product.

We will generally assume that the uptake functions are *unimodal* in the following sense. The functions

$$c \mapsto \tilde{g}(c), \quad c \mapsto \tilde{f}(1 - c, c) - Ec ,$$

and for fixed  $c$ ,

$$s \mapsto \tilde{f}(s, c)$$

have at most one critical point, a local maximum, and nonzero derivatives everywhere else. In particular,

$$f_s(0, 0) > 0, \quad g_c(0) > 0 .$$

We also assume  $\tilde{f}(S^0, 0) > 0$  and  $\tilde{g}\left(\frac{K_2}{K_1}S^0\right) > 0$ . Kinetics will be called *monotone*, if

$$\tilde{f}_s(s, c) > 0, \quad \tilde{g}_c(c) > 0 \text{ for all } s \in [0, S^0], \quad c \in \left[0, \frac{K_2}{K_1}S^0\right] ,$$

and *non-monotone* otherwise.

*Examples.* The standard example for monotone kinetics is Michaelis-Menten

$$\tilde{f}(s) = \frac{As}{\mu + s}, \quad \tilde{g}(c) = \frac{Bc}{\lambda + c}, \quad A, B, \lambda, \mu > 0 ,$$

where  $A$  and  $B$  represent the turnover rates at saturation with substrate,  $\mu$  and  $\lambda$  the half-saturation constants. Kreikenbohm and Bohl [17] and Wilkinson [30] both assume Michaelis-Menten kinetics for  $\tilde{g}$ , and for  $\tilde{f}$  the modified expressions

$$\tilde{f}(s, c) = \frac{As}{(\mu + s)(v + c)}, \quad A, \mu, v > 0 \quad (\text{Wilkinson}),$$

$$\tilde{f}(s, c) = \frac{A(s - \alpha c)}{\mu + s + vc}, \quad A, \alpha, \mu, v > 0 \quad (\text{Kreikenbohm and Bohl}).$$

Linearising  $\tilde{f}$  and  $\tilde{g}$  at zero, we obtain

$$\tilde{f}(s, c) = \frac{A}{\mu}(s - \alpha c), \quad \tilde{g}(c) = \frac{B}{\lambda}c$$

where the constants  $A, B, \lambda, \mu$ , and  $\alpha$  are as above, with  $\alpha = 0$  for Michaelis-Menten kinetics and the Wilkinson model. In the special case  $E = 0$  (no toxic effects of the product), the system can be reduced to a Volterra-type model (see §§6, 7).

The model equations generate a flow on  $\mathbf{R}^4$ , which depends continuously on initial values, time and parameters. As state space we use the set

$$\mathbf{X} := \{(s, u, c, v) \in \mathbf{R}^4 \mid u, v \geq 0\}$$

which is invariant under the flow generated by the equations. The meaningful non-negative states form a positively invariant subset.

*Scaling.* Fix a unit of time  $T > 0$  and define

$$\begin{aligned} s_{\text{new}} &:= \frac{1}{S^0} \cdot s_{\text{old}} & u_{\text{new}} &:= \frac{K_1}{S^0} \cdot u_{\text{old}} \\ t_{\text{new}} &:= \frac{1}{T} \cdot t_{\text{old}} & c_{\text{new}} &:= \frac{K_1}{K_2 S^0} \cdot c_{\text{old}} & v_{\text{new}} &:= \frac{K_1 K_3}{K_2 S^0} \cdot v_{\text{old}} \end{aligned}$$

to obtain

$$\begin{aligned} \dot{s} &= D(1 - s) - f(s, c)u \\ \dot{u} &= -Du + f(s, c)u - Ecu \\ \dot{c} &= -Dc + f(s, c)u - g(c)v \\ \dot{v} &= -Dv + g(c)v \end{aligned}$$

where

$$\begin{aligned} D_{\text{new}} &= T \cdot D_{\text{old}} & f_{\text{new}}(s, c) &= T \cdot f_{\text{old}}\left(S^0 \cdot s, \frac{K_2 S^0}{K_1} \cdot c\right) \\ E_{\text{new}} &= \frac{TK_2 S^0}{K_1} \cdot E_{\text{old}} & g_{\text{new}}(c) &= T \cdot g_{\text{old}}\left(\frac{K_2 S^0}{K_1} \cdot c\right). \end{aligned}$$

The scaled equations are dimensionless.

*Remark.* Varying  $D$  in the original system corresponds to varying  $D$  in the scaled system. If we simultaneously replace  $D$  and  $S^0$  with  $\lambda D$  and  $\lambda S^0$  and rescale time with  $\lambda^{-1}T$ , then in the scaled equations,  $D$  is unchanged, while  $f(s, c)$  and  $g(c)$  are changed to  $\lambda^{-1}f(\lambda s, \lambda c)$  and  $\lambda^{-1}g(\lambda c)$ . By unimodality, the functions will become monotone for  $s, c < 1$  when  $\lambda$  is sufficiently small, and converge to their linearisations at zero in the limit  $\lambda \rightarrow 0$ . Together with the uniform asymptotic bounds which we show below, this means that we can force the kinetics to be monotone or even approximately linear by choosing  $D$  and  $S^0$  small enough. It is

interesting to note that for the Wilkinson model, the inhibitory effect of the product vanishes as  $\lambda$  becomes small, whereas  $\alpha$  in the Kreikenbohm-Bohl model and the toxicity parameter  $E$  are not affected.

There are two natural mass balances. The first relates consumption of substrate with conversion into the metabolic product and with the biomass of the consumer. This leads to a differential equation. The second relates consumption of the substrate with increase in biomass of the producer. Unless the toxicity parameter  $E$  is zero, this balance is imperfect, leading only to a differential inequality. We use them in the following lemma to reduce the dimension of the system.

**Lemma** (Attracting subspaces) *The hyperplane*

$$\{(s, u, c, v) \in \mathbf{X} \mid s + c + v = 1\}$$

*is invariant and globally exponentially attracting in  $\mathbf{X}$ . The closed positive cone corresponds to*

$$\{(u, c, v) \in \mathbf{R}^3 \mid u, v \geq 0, c + v \leq 1\} .$$

*Moreover, the set*

$$\{(u, c, v) \in \mathbf{R}^3 \mid 0 \leq u, c, v \leq c + v \leq 1\}$$

*is positively invariant and contains the global attractor for the positive cone.*

*If  $E = 0$ , the plane*

$$\{(s, u, c, v) \in \mathbf{X} \mid s + c + v = 1, s + u = 0\}$$

*is invariant and globally exponentially attracting. The intersection with the positive cone corresponds to the triangle*

$$\{(u, v) \in \mathbf{R}^2 \mid 0 \leq v \leq u \leq 1\} .$$

*Proof.* For a solution  $\mathbf{x}(t) = (s(t), u(t), c(t), v(t))_{t \in \mathbf{R}_0^+}$  in  $\mathbf{X}$  define

$$z(t) := s(t) + c(t) + v(t)$$

$$w(t) := s(t) + u(t) .$$

These satisfy the differential equations

$$\dot{z}(t) = D(1 - z(t))$$

$$\dot{w}(t) = D(1 - w(t)) - Ec(t)u(t) .$$

Integration gives

$$z(t) = 1 + (z(0) - 1)e^{-Dt} ,$$

and for  $E = 0$

$$w(t) = 1 + (z(0) - 1)e^{-Dt} .$$

If  $E$  is positive, then for  $c(0)$  nonnegative,  $c(t)$  will be nonnegative for positive times. Integrating the differential inequality we obtain

$$w(t) \leq 1 + (z(0) - 1)e^{-Dt} .$$

The following lemma will be used to prove extinction and persistence results in §5.

**Lemma** (Positivity and bounds) *For every solution  $\mathbf{x}(t) = (s(t), u(t), c(t), v(t))_{t \in \mathbf{R}}$  in  $\mathbf{X}$  which does not converge to  $(1, 0, 0, 0)$  there is a positive time  $T$ , such that for  $t > T$ :*

- a)  $s(t) > 0$
- b)  $c(t) > 0$
- c)  $s(t) < 1$
- d)  $f(s(t), c(t)) > 0$
- e)  $s(t) + u(t) < 1$
- f)  $c(t) + v(t) - u(t) > 0$ .

*In particular, solutions are uniformly asymptotically bounded for positive time. Moreover the attractor for the positive cone is in fact the global attractor for  $\mathbf{X}$ , and for all considerations of  $\omega$ -limit sets we may replace  $f$  and  $g$  by  $\tilde{f}$  and  $\tilde{g}$  whenever it seems convenient.*

*Proof.* Take  $\mathbf{x}(t) = (s(t), u(t), c(t), v(t))_{t \in \mathbf{R}}$  as above.

a) As long as  $s(t) \leq 0$ , it satisfies

$$\dot{s}(t) = D(1 - s(t)).$$

b) If  $c(t) \leq 0$  for all positive times, then  $g(c(t)) \equiv 0$  by the positivity assumptions, so

$$\begin{aligned}\dot{c}(t) &= -Dc(t) + f(s(t), c(t))u(t) \geq -Dc(t) \\ \dot{v}(t) &= -Dv(t)\end{aligned}$$

and  $c(t)$  and  $v(t)$  converge ( $t \rightarrow \infty$ ) to zero,  $s(t)$  to one. Because  $c(t)$  converges monotonically,  $\dot{c}(t)$  goes to zero as well, so  $\dot{u}$  has to become eventually negative unless  $u = 0$ .

c) From

$$\dot{s}(t) \geq D(1 - s(t))$$

it follows that  $\overline{\lim}_{t \rightarrow \infty} s(t) \leq 1$ . If always  $s(t) \geq 1$ , then because of a)  $\lim_{t \rightarrow \infty} s(t) + c(t) + v(t) = 1$ , b)  $\underline{\lim}_{t \rightarrow \infty} c(t) \geq 0$ , so necessarily  $c(t) \rightarrow 0$  and  $v(t) \rightarrow 0$ . Convergence of  $u$  follows as in b).

d) So long as  $f(s(t), c(t)) = 0$ ,  $s(t)$ ,  $u(t)$ , and  $c(t)$  satisfy

$$\begin{aligned}\dot{s}(t) &= D(1 - s(t)) \\ \dot{c}(t) &= -Dc(t).\end{aligned}$$

Integrating we obtain  $s(t) \rightarrow 1$  and  $c(t) \rightarrow 0$ . For  $t$  large enough,  $u$  and  $v$  satisfy the differential inequalities

$$\begin{aligned}\dot{u}(t) &\leq -D/2u(t) \\ \dot{v}(t) &\leq -D/2v(t).\end{aligned}$$

For a solution not approaching the trivial equilibrium,  $1 - s(t)$  and  $c(t)$  will eventually become positive, so the solution must enter the set

$$\{(s, u, c, v) \in \mathbf{X} \mid 0 < s < 1, c > 0, f(s, c) > 0\}$$

which is positively invariant because of a)–c) and since, for  $s < 1$ ,  $c > 0$ ,

$$\tilde{f}(s, c) = 0 \Rightarrow \frac{d}{dt}\tilde{f}(s, c) = \tilde{f}_s(s, c) \cdot D(1-s) + \tilde{f}_c(s, c) \cdot (-Dc) > 0$$

e) and f) are proved similarly to a)–c). □

### 3 The equilibria

For the discussion of the equilibria we restrict to the exponentially attracting hyperplane  $\{(s, u, c, v) \mid s + c + v = 1\}$ . The flow is given by

$$\begin{aligned} \dot{u} &= u(-D + f(1-c-v, c) - Ec) \\ \dot{c} &= -Dc + f(1-c-v, c)u - g(c)v \\ \dot{v} &= v(-D + g(c)). \end{aligned}$$

Equilibria are nonnegative solutions of

$$\begin{aligned} u(-D + f(1-c-v, c) - Ec) &= 0 \\ -Dc + f(1-c-v, c)u - g(c)v &= 0 \\ v(-D + g(c)) &= 0. \end{aligned}$$

We will use  $D$  as bifurcation parameter and keep everything else fixed.

There are three types of critical points: The ‘empty’ chemostat, boundary equilibria in which only the first species survives, and positive equilibria where both species coexist.

The trivial equilibrium  $(0, 0, 0)$  exists for all parameter values. The matrix of the linearisation

$$J(0, 0, 0) = \begin{pmatrix} -D + f(1, 0) & 0 & 0 \\ f(1, 0) & -D & 0 \\ 0 & 0 & -D \end{pmatrix}$$

has the eigenvalues  $-D$  (double) and  $-D + f(1, 0)$  (simple), so decreasing  $D$  stability is lost to a branch of boundary equilibria at  $D = f(1, 0)$ .

Boundary equilibria are positive solutions of

$$\begin{aligned} f(1-c, c) - Ec &= D \\ u &= \frac{c}{1 + E/Dc} \end{aligned}$$

with  $v = 0$ . The linearization matrix is

$$J = \begin{pmatrix} 0 & -(f_s - f_c + E)u & * \\ f(1-c, c) & -D - (f_s - f_c)u & * \\ 0 & 0 & -D + g(c) \end{pmatrix}.$$

There are two branches. The first has  $f_s - f_c + E > 0$ . It exists for  $0 < D < D^0 := \max_{c \in [0, 1]} f(1-c, c) - Ec$ . It is always locally stable within the  $u$ - $c$ -plane, since

$$(f_s - f_c + E)u = (f_s - f_c)u + E \frac{Dc}{D + Ec} \leq (f_s - f_c)u + D,$$

so the determinant of the restriction is positive, the trace negative. The second branch has  $f_s - f_c - E < 0$ . It exists for  $f(1, 0) < D < D^0$  which is a nonempty interval



only if  $f$  is not monotonic. It is unstable, because the determinant of the restriction is negative.

Boundary equilibria bifurcate from the trivial equilibrium at  $D = f(1, 0)$ . If  $f$  is not monotonic the two branches join in a saddle-node bifurcation at  $D = D^0$ . Whenever  $g(c) - D$  changes sign, a branch of positive equilibria ends. Periodic solutions can never bifurcate from boundary equilibria because the linearisation cannot have purely imaginary eigenvalues.

Positive equilibria are positive solutions of

$$\begin{aligned} g(c) &= D \\ f(1 - c - v, c) &= D + Ec \\ u &= \frac{c + v}{1 + E/Dc} . \end{aligned}$$

For unimodal kinetics there is at most one solution for each sign combination of  $f_s$  and  $g_c$ . The characteristic polynomial  $\chi(\lambda) = a_0 \lambda^3 + a_1 \lambda^2 + a_2 \lambda + a_3$  of the linearization matrix

$$J = \begin{pmatrix} 0 & -(f_s - f_c + E)u & -f_s u \\ f(1 - c - v, c) & -D - (f_s - f_c)u - g_c v & -D - f_s u \\ 0 & g_c v & 0 \end{pmatrix}$$

has coefficients

$$\begin{aligned} a_0 &= 1 \\ a_1 &= D + (f_s - f_c)u + g_c v \\ a_2 &= g_c v(D + f_s u) + f(f_s - f_c + E)u \\ a_3 &= f f_s u g_c v . \end{aligned}$$

Using the Routh-Hurwitz criterion the number of eigenvalues with positive real part is zero, if  $f_s$  and  $g_c$  are both positive, one or three, if they have different signs, and zero or two, if they are both negative. If there are positive equilibria, one of them has both  $f_s$  and  $g_c$  positive; it is always stable, and both populations reach the maximal densities possible in an equilibrium. Positive equilibria bifurcate from boundary equilibria when  $v = 0$  in a solution of the above equations. Two branches join in a saddle-node bifurcation when  $f_s$  or  $g_c$  changes sign. A pair of eigenvalues crosses the imaginary axis when  $a_1 a_2 - a_3$  changes sign and  $a_1 a_3$  is positive; this can happen only on branches where at least one of  $f_s$  and  $g_c$  is negative. We will discuss bifurcation of periodic solutions in the next section.

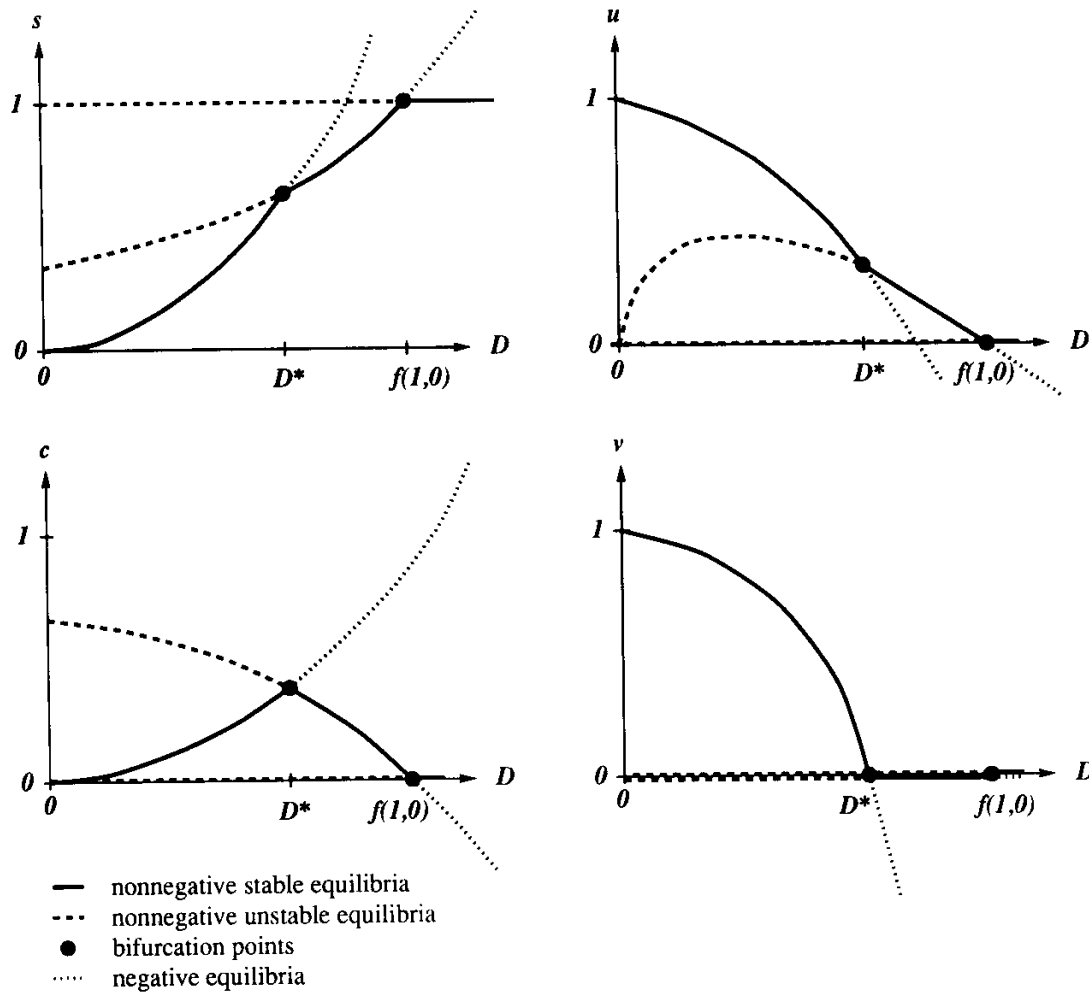
The bifurcation diagram for monotone kinetics is given by Fig. 1. For each parameter value there is at most one equilibrium of each type, exactly one of which is stable. The positive equilibrium exists for  $D < D^*$ , defined by

$$f(1 - c^*, c^*) - Ec^* = g(c^*) =: D^* ,$$

and it is always asymptotically stable. The boundary equilibrium exists for  $D < f(1, 0)$  and is always stable within the  $u - c$  plane; the transversal direction is stable for  $D > D^*$ , unstable otherwise.

#### 4 Periodic solutions

If the kinetics of nutrient uptake is not monotone, then periodic solution may bifurcate from positive equilibria. One can give sufficient conditions for a pair of



**Fig. 1.** Bifurcation diagram of equilibria for monotone kinetics ( $E > 0$ ). For large  $D$ , there is complete washout. Lowering  $D$ , stability is lost to a boundary equilibrium at  $D = f(1, 0)$ , and in secondary bifurcation to a positive equilibrium at  $D^*$

eigenvalues to cross the imaginary axis along a branch of equilibria as  $D$  is varied (see [5]). We will show, that Hopf bifurcation does take place for some choices of the uptake functions, and that the bifurcating periodic solutions may be stable or unstable.

There are at least four ways to show bifurcation of periodic solutions. First, there are the methods of *global* bifurcation theory. Under a nondegeneracy condition, a pair of eigenvalues crossing the imaginary axis implies the existence of a continuum of periodic solutions which is unbounded in either amplitude or period (see Fiedler [9, 10]). Unfortunately, although the transversality condition is *generically* satisfied for general right hand sides, it is not obvious that this is true in the restricted class of right hand sides corresponding to our model. As the condition involves Floquet multipliers of periodic solutions, there is no hope we can calculate it for our system. No statement is made about stability.

Second, *local* analysis of the Hopf bifurcation proves difficult for our example. Stability and transversality are determined by third order terms in the Taylor expansions around the bifurcation point (Marsden and McCracken [19]), that is, in our case, by third derivatives of the uptake functions at a point which is defined implicitly as the solution to a system of nonlinear equations.

Third, reformulating the question as “For what kinds of right hand sides will there be Hopf bifurcation when  $D$  is varied?” we think of it as a two-parameter problem. One parameter is  $D$ , as before; the second parametrises smoothly a family of uptake functions  $f$  and  $g$ , also the toxicity parameter  $E$  may depend on it. We will formulate transversality conditions later. We will restrict our discussion to a neighborhood of Bogdanov-Takens singularities which show up in our model for special right hand sides.

Finally, Hopf Bifurcation and periodic solutions can be found by numerical methods. The Bogdanov-Takens singularities we find here give an indication in which regions of the state and parameter space one can expect to find periodic solutions. In [5] we give some geometric criteria on the graphs of  $f$  and  $g$  for Hopf bifurcation and Bogdanov-Taken singularities in the case that  $f = f(s)$ .

In two-parameter families, Bogdanov-Takens singularities arise naturally as the endpoints of curves of Hopfs bifurcation points. For a detailed discussion of Bogdanov-Takens singularities in general we refer to Bogdanov [3], Takens [28] or [1, 8, 12]. We will follow Bogdanov’s presentation and in particular use his normal form.

For our present purposes, Bogdanov’s results can be paraphrased as follows. Given a sufficiently smooth two-parameter family of differential equations in the plane. Assume that for the parameters both zero, the origin is an equilibrium such that the linearisation has a geometrically double, algebraically simple eigenvalue zero. By linear transformation, we can assume that the equation has the form

$$\begin{aligned}\dot{x} &= y + \frac{1}{2}H_{11}x^2 + H_{12}xy + \frac{1}{2}H_{22}y^2 + o(x^2 + y^2) \\ \dot{y} &= \frac{1}{2}K_{11}x^2 + K_{12}xy + \frac{1}{2}K_{22}y^2 + o(x^2 + y^2).\end{aligned}\quad (1)$$

**Theorem** (Bogdanov [3]) (see [5]) *If the right hand side of the differential equation (1) is four times continuously differentiable and the coefficient  $K_{11} \neq 0$ , then there is a transformation ( $C^4$  in  $x$  and  $y$ , depending continuously on the parameters) such that the second order terms take the normal form*

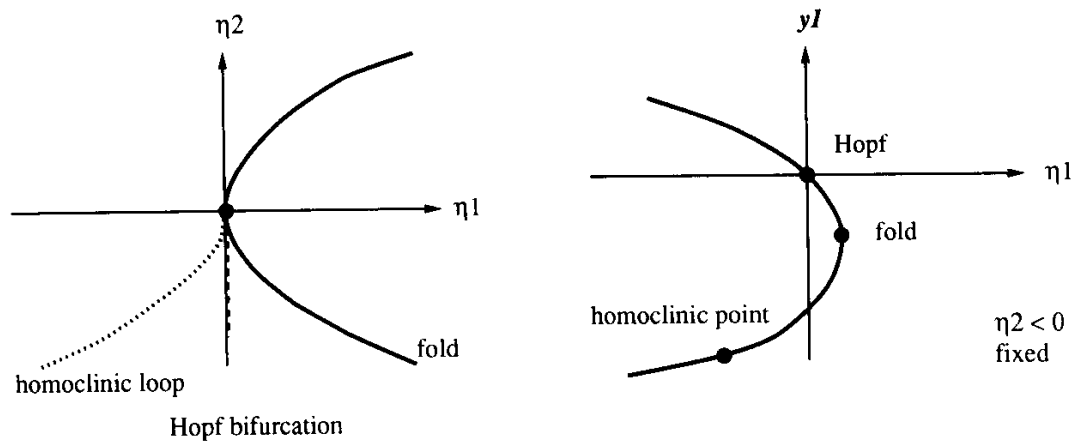
$$\begin{aligned}\dot{x} &= y + o(x^2 + y^2) \\ \dot{y} &= \eta_1 + \eta_2x + x^2 + qxy + o(x^2 + y^2)\end{aligned}\quad (2)$$

*uniformly for the parameters near  $(0, 0)$ , where  $q = (H_{11} + K_{12})/K_{11}$  and  $\eta$  is a continuous function of the original parameters.*

**Theorem** (Bogdanov [3]) *The bifurcation diagram for the system in the normal form*

$$\begin{aligned}\dot{x} &= y \\ \dot{y} &= \eta_1 + \eta_2x + x^2 + qxy\end{aligned}\quad (3)$$

*with  $q \neq 0$  is given by Fig. 2. Crossing the fold from right to left, a pair of equilibria is generated in a saddle-node bifurcation. Periodic solutions exist for  $\eta_2 < 0$  between the curves marking Hopf bifurcation and homoclinic loops. They are*



**Fig. 2.** Bifurcation diagram for Bogdanov-Takens singularity in normal form. Lowering  $\eta_2$ , a saddle-node bifurcation takes place when crossing the fold. A unique periodic solution is generated in the Hopf bifurcation and ends in the homoclinic loop

unique and stable, if  $q < 0$ , and unstable, if  $q > 0$ . The flow for  $q > 0$  is obtained from the system for  $q < 0$  by the time reversing transformation  $t \mapsto -t$ ,  $y \mapsto -y$ .

The bifurcation diagram and dynamics of the perturbed system (2) are equivalent to the normal form (3) for parameter values  $\eta \neq (0, 0)$  provided  $q \neq 0$ . The same is true for the original system, if the new parameters are differentiable functions of the old parameters whose derivative at  $(0, 0)$  has full rank.

*Remark.* (i) The theorem implies that the bifurcation diagram and dynamics near a Bogdanov-Takens singularity are, under nondegeneracy conditions, determined by the Taylor expansion of the vector field up to second order. In particular, the stability condition for the periodic solutions contains only quadratic terms. These terms are calculated from the original equation by projection onto the tangent space of the invariant manifold. The third order terms determining transversality and stability in the Hopf bifurcation cannot be calculated by linear projection.

(ii) The homoclinic loop is part of the *local* bifurcation diagram at the Bogdanov-Takens singularity. If only one parameter is varied, the bifurcation of periodic solutions from a homoclinic loop is not a local phenomenon.

In our system, the conditions for a positive equilibrium  $(s^o, u^o, c^o, v^o)$  at parameter values  $(D^o, \mu^o)$  to be a Bogdanov-Takens singularity are the following.

$$\begin{array}{ll} \text{a) } \begin{array}{l} g_c^o = 0 \\ f_s^o + \tilde{E}_s^o = 0 \\ \tilde{E}^o = E^o - f_c^o \neq 0 \\ \lambda_3 = -(D^o - E^o u^o) \neq 0 \end{array} & \text{or b) } \begin{array}{l} f_s^o = 0 \\ D^o g_c^o v^o + f^o u^o (f_s^o + \tilde{E}^o) = 0 \\ \tilde{E}^o = E^o - f_c^o \neq 0 \\ \lambda_3 = -(D^o - f_c^o u^o - g_c^o v^o) \neq 0. \end{array} \end{array}$$

The first two conditions ensure, that the linearisation has a double eigenvalue zero. The third condition says that there is only one eigenvector associated with it. The other two eigenvalues of the linearisation are

$$\text{a) } \begin{array}{l} \lambda_3 = -(D^o - E^o u^o) \\ \lambda_4 = -D^o \end{array} \quad \text{b) } \begin{array}{l} \lambda_3 = -(D^o - f_c^o u^o + g_c^o v^o) \\ \lambda_4 = -D^o. \end{array}$$

Under the above assumptions, there exists a local two-dimensional center manifold at the singularity, that is a locally invariant manifold tangent to the generalised

null space (see for example Vanderbauwhede [31]). The flow on this manifold together with the signs of  $\lambda_3$  and  $\lambda_4$  determines the full flow for parameters and initial values in a neighborhood up to conjugacy. The manifold is exponentially attracting if  $\lambda_3 < 0$ .

We calculate the equation of the restriction to this manifold. The projection onto the generalised null space along the complementary eigenspace can be written as

$$P(s, u, c, v) = ((s, u, c, v), \Psi_1) \Phi_1 + ((s, u, c, v), \Psi_2) \Phi_2 = x \Phi_1 + y \Phi_2$$

where

$$\text{a) } \quad \Phi_1 = \tilde{E}^o u^o \begin{pmatrix} -f^o \\ -\lambda_3 \\ f^o \\ 0 \end{pmatrix} \quad \Phi_2 = \begin{pmatrix} f_c^o u^o \\ \tilde{E}^o u^o \\ -D^o + \tilde{E}^o u^o \\ -\lambda_3 \end{pmatrix}$$

$$\text{b) } \quad \Phi_1 = \tilde{E}^o u^o \begin{pmatrix} f^o \\ -D^o \\ 0 \\ -f^o \end{pmatrix} \quad \Phi_2 = \begin{pmatrix} -D^o - \lambda_3 \\ 0 \\ D^o \\ \lambda_3 \end{pmatrix}$$

span the generalised null space of the linearisation at zero, and

$$\text{a) } \quad \Psi_1 = \lambda_3^{-1} \begin{pmatrix} (D^o)^{-1} \\ -(\tilde{E}^o u^o)^{-1} \\ (D^o)^{-1} \\ (D^o)^{-1} \end{pmatrix} + \lambda_3^{-1} \Psi_2 \quad \Psi_2 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ -\lambda_3^{-1} \end{pmatrix}$$

$$\text{b) } \quad \Psi_1 = (D^o)^{-1} \begin{pmatrix} 0 \\ -(\tilde{E}^o u^o)^{-1} \\ -\lambda_3^{-1} \\ 0 \end{pmatrix} + \lambda_3^{-1} f^o \Psi_2 \quad \Psi_2 = \lambda_3^{-1} \begin{pmatrix} 0 \\ -(D^o)^{-1} f^o \\ 0 \\ 1 \end{pmatrix}$$

span the generalised null space of the adjoint, and the orthogonality relations

$$(\Psi_i, \Phi_j) = \delta_{ij} \text{ for } i, j = 0, 1$$

are satisfied.

We calculate for the coefficients

$$\text{a) } \quad K_{11} = -\lambda_3^{-1} (f^o)^2 (\tilde{E}^o u^o)^2 g_{cc}^o v^o$$

$$H_{11} + K_{12} = -\lambda_3^{-1} (f^o)^2 \tilde{E}^o u^o (f_{ss}^o - 2f_{sc}^o + f_{cc}^o) u^o - \lambda_3^{-2} f^o \tilde{E}^o u^o$$

$$(f^o \tilde{E}^o u^o - \lambda_3 (D^o - \tilde{E}^o u^o)) g_{cc}^o v^o$$

$$\text{b) } \quad K_{11} = -\lambda_3^{-1} (D^o)^{-1} (f^o)^3 (\tilde{E}^o u^o)^2 f_{ss}^o u^o$$

$$H_{11} + K_{12} = -(\lambda_3)^{-2} (D^o)^{-2} (f^o)^4 (\tilde{E}^o u^o)^2 f_{ss}^o u^o$$

$$-\lambda_3^{-1} (f^o)^2 \tilde{E}^o u^o f_{sc}^o u^o + \lambda_3^{-1} D^o f^o (\tilde{E}^o)^2 u^o c^o (v^o)^{-1}.$$

In a), varying  $D$  for fixed  $\mu$  near  $D = g(\hat{c}(\mu), \mu)$ , saddle-node bifurcation takes place, which is nondegenerate for  $g_{cc}^o \neq 0$ . The map  $D \mapsto \eta(D, \mu^o)$  is transversal to the fold in Fig. 2, so we can identify  $D - D^o$  with the parameter  $\eta_1$ , and parametrise

the fold over  $\mu$ . Similarly in b), if  $f_{ss}^o \neq 0$  and  $\tilde{E}^o + g_c^o \neq 0$ , we can use  $D - D^o$  as parameter  $\eta_1$  near the singularity and parametrise the fold over  $\mu$ .

Identifying  $\eta_2$  in normal form as a constant multiple of the nonzero eigenvalue  $\lambda_2$  on the fold, we need to check that the total derivative of this eigenvalue along the fold with respect to  $\mu$  is nonzero. In the fold,

$$\begin{aligned} \text{a)} \quad & \lambda_2 \lambda_3 = f u (f_s - f_c + E) \\ \text{b)} \quad & \lambda_2 \lambda_3 = D g_c v + f u (-f_c + E) \end{aligned}$$

using the expressions for the coefficients of the characteristic polynomial from §3. Along the fold, all the variables including  $D$  and  $E$  and all the functions and their partial derivatives can be written as functions of  $\mu$ . The total derivative  $d\lambda_2/d\mu$  is nonzero under a nondegeneracy condition on the partial derivatives of  $f$ ,  $g$ ,  $f_s$ ,  $f_c$ ,  $g_c$ , and  $E$  with respect to  $\mu$ .

We summarise the transversality conditions

$$\begin{aligned} \text{a)} \quad & \begin{aligned} & g_{cc}^o \neq 0 \\ & H_{11} + K_{12} \neq 0 \\ & \frac{d\lambda_2}{d\mu} \Big|_{(D^o, \mu^o)} \neq 0 \end{aligned} \\ \text{b)} \quad & \begin{aligned} & f_{ss}^o \neq 0 \\ & H_{11} + K_{12} \neq 0 \\ & \frac{d\lambda_2}{d\mu} \Big|_{(D^o, \mu^o)} \neq 0, \tilde{E}^o + g_c^o \neq 0 \end{aligned} \end{aligned}$$

where the first two are the transversality conditions on the coefficients in the Bogdanov-Takens singularity, the third guarantees that  $D$  and  $\mu$  are a set of independent parameters.

Finally, the conditions for stability of the bifurcating periodic solutions are

$$\begin{aligned} \lambda_3 &< 0 \\ H_{11} + K_{12} &> 0 \end{aligned}$$

where the first is the condition for the local center manifold to be exponentially attracting. Note that this implies that the determinant of the linearisation is positive, so the Hopf bifurcation takes place from the branch of equilibria where  $f_s$  and  $g_c$  are both negative. By unimodality and the transversality condition from above,  $K_{11} < 0$ , so the second condition is exactly what is needed to make  $q$  in the normal form (3) negative.

Hopf bifurcation and bifurcation of periodic solutions from homoclinic loops take place varying  $D$  for right hand sides near the singularity. With the possible exception of the flow for the parameters at the critical value  $D = D^o$ ,  $\mu = \mu^o$  ( $\eta = 0$ ), the bifurcation diagram and the dynamics are equivalent to the system in normal form as shown in Fig. 2.

The second derivatives of  $f$  and  $g$  enter with opposite signs into the formula for  $H_{11} + K_{12}$ . Consequently, periodic solutions may be stable or unstable, depending on the shape of the uptake functions near the singularity.

If  $E = 0$ , the local center manifold coincides with the two-dimensional exponentially attracting plane. No spectral projection is necessary. Using  $E = 0$ ,  $u = c + v = 1 - s$  on this plane,  $f^o = D^o$ , and  $\lambda_3 = \lambda_4 = -D^o$  at the singularity, the formulas for the coefficients simplify to

$$\begin{aligned} \text{a)} \quad & \begin{aligned} K_{11} &= D^o (f_c^o u^o)^2 g_{cc}^o v^o \\ H_{11} + K_{12} &= -D^o f_c^o u^o (f_{ss}^o - 2f_{sc}^o + f_{cc}^o) u^o + D^o f_c^o u^o g_{cc}^o v^o \end{aligned} \\ \text{b)} \quad & \begin{aligned} K_{11} &= D^o (f_c^o u^o)^2 f_{ss}^o u^o \\ H_{11} + K_{12} &= -(f_c^o u^o)^2 f_{ss}^o u^o - D^o f_c^o u^o f_{sc}^o u^o - D^o (f_c^o)^2 u^o c^o (v^o)^{-1}. \end{aligned} \end{aligned}$$

As in the general case, periodic solutions may be stable or unstable.

We will show in §6 that there are no periodic solutions for  $D$  small enough.

In the commensal case ( $f = f(s)$ ,  $E = 0$ ), where the growth of the producer is not influenced by the consumer at all, the system decouples into two first order equations. It is easy to show that all trajectories approach equilibrium, no matter what  $f$  and  $g$  look like. For unimodal  $f$  and  $g$ , there is always exactly one stable equilibrium attracting the solutions through almost all initial values.

## 5 The flow on the boundary. Persistence and extinction

In this section, we discuss the two single-species subsystems of our model, assuming monotone kinetics. With the help of a theorem by Butler, Freedman, and Waltman [6, 7] we find critical values of the dilution rate determining survival and extinction of one or both species. We interpret “survival” as *uniform persistence*, which indicates, that the population cannot become extinct by an arbitrarily small perturbation or fluctuation.

Naturally, the consuming species cannot survive in pure culture. On trajectories with  $u = 0$  in the initial point

$$\begin{aligned}\dot{s} &= D(1 - s) \\ \dot{c} &= -Dc - g(c)v \\ \dot{v} &= -Dv + g(c)v\end{aligned}$$

so  $\lim_{t \rightarrow \infty} s(t) = 1$ , and as  $c$  must become eventually positive, all solutions approach the trivial equilibrium. This means that under the given conditions, the consumer is an obligate mutualist.

The producing species survives in pure culture, provided that the dilution rate is not too large, so the association is facultative for this species. We will show later in this section that for  $D > f(1, 0)$ , all solutions converge to the trivial equilibrium. For  $D < f(1, 0)$ , the stable boundary equilibrium attracts all solutions with  $u(0) > 0$ ,  $v(0) = 0$ . In this boundary equilibrium,  $u \rightarrow 0$  as  $D \rightarrow 0$ , if  $E > 0$ , and to a positive limit otherwise, showing that in the limit for dilution rates, the producer becomes dependent on the consumer.

To prove this, consider the intersection with the exponentially attracting hyperplane  $\{s + c + v = 1\}$ . The flow on this surface is generated by

$$\begin{aligned}\dot{u} &= -Du + f(1 - c, c)u - Ecu \\ \dot{c} &= -Dc + f(1 - c, c)u.\end{aligned}$$

We transform

$$\begin{aligned}y &= \log u \\ z &= c\end{aligned}$$

to obtain

$$\begin{aligned}\dot{y} &= -D + f(1 - z, z) - Ez \\ \dot{z} &= -Dz + f(1 - z, z) \exp y.\end{aligned}$$

As the flow induced by the first set of equations is bounded and strongly persistent (see lemma below), all solutions of the transformed system stay bounded for positive times, so  $\omega$ -limit sets of points are nonempty. The divergence of the right hand

side is strictly negative; by the Bendixson criterion for planar flows there are no periodic orbits and no loops. One branch of the unstable manifold of  $(0, 0)$  points into the positive cone; applying the Poincaré-Bendixson theorem we obtain a unique connection to the nontrivial equilibrium.

**Lemma** *We have the following persistence results. For  $D < f(1, 0)$*

$$u(0) > 0 \Rightarrow \liminf_{t \rightarrow \infty} u(t) > 0$$

and for  $D < D^*$

$$v(0) > 0 \Rightarrow \liminf_{t \rightarrow \infty} v(t) > 0.$$

*Proof.* In the given parameter ranges, the planes  $\{u = 0\}$  and  $\{v = 0, u > 0\}$  are the global stable manifolds of the trivial and boundary equilibrium, respectively.  $\square$

In the next two propositions, we find the following threshold values for  $D$ . If the dilution rate  $D$  is larger than  $f(1, 0)$ , then both species are completely washed out. For all smaller values of  $D$  the producing species is uniformly persistent. For  $D$  between  $f(1, 0)$  and the next threshold value,  $D^{**}$ , the second species always dies out, and the producer survives in a stable equilibrium. For  $D$  less than the critical value,  $D^*$ , below which the two species coexist in an equilibrium, we prove uniform persistence for both. If  $E = 0$ ,  $D^* = D^{**}$ , we will show in the next section, that all solutions converge to equilibrium. In general, however,  $D^{**} > D^*$ , we do not know what happens for  $D$  between these two values, and we were not able to show convergence to equilibrium for  $D < D^{**}$ .

**Proposition 1** (Extinction of one or both species) *For  $D \geq f(1, 0)$  the trivial equilibrium  $(1, 0, 0, 0)$  is globally attracting in  $\mathbf{X}$ .*

*If  $D \geq D^{**}$  defined by*

$$f(1 - c^{**}, c^{**}) = g(c^{**}) =: D^{**}$$

*then for every solution  $\mathbf{x}(t) = (s(t), u(t), c(t), v(t))$  in  $\mathbf{X}$*

$$\lim_{t \rightarrow \infty} v(t) = 0.$$

*So for  $D^{**} \leq D < f(1, 0)$ , a solution converges to the trivial equilibrium if  $u(0) = 0$ , and to the boundary equilibrium otherwise.*

*Proof.* Take  $D \geq f(1, 0)$  and assume

$$\lim_{t \rightarrow \infty} \mathbf{x}(t) \neq (1, 0, 0, 0),$$

this implies  $u(0) > 0$ . We showed in the positivity lemma in §2 that on such a trajectory there exists a time  $T$  such that

$$t > T \Rightarrow s(t) < 1, c(t) > 0.$$

$u(t)$  satisfies for  $t > T$



$$\begin{aligned}\dot{u}(t) &= u(t)(-D + f(s, c) - Ec) \\ &< u(t)(-D + \max_{c \in [0, 1]} f(1 - c, c)) \\ &\leq 0.\end{aligned}$$

By integration

$$\lim_{t \rightarrow \infty} u(t) = 0.$$

The trivial equilibrium is globally attracting within the hyperplane  $\{u = 0\}$ , so

$$\omega(\mathbf{x}) = \{(1, 0, 0, 0)\}.$$

Existence of  $D^{**}$  follows with the intermediate value theorem from

$$f(0, 1) - g(1) < 0, \quad f(1, 0) - g(0) > 0$$

and uniqueness from the monotonicity properties of  $f$  and  $g$ . For  $D \geq D^{**}$  consider the restriction to the exponentially attracting hyperplane

$$\{(s, u, c, v) \mid s + c + v = 1; s, u, c, v \geq 0\}.$$

Define the set

$$\mathbf{B} := \{(u, c, v) \in \mathbf{R}^3 \mid 0 \leq u, c, v \leq c + v \leq 1; g(c) \leq D\}.$$

$\mathbf{B}$  is positively invariant, because

$$g(c) \geq D > D^{**}, \quad 0 \leq u, c, v \leq c + v \leq 1$$

implies

$$\begin{aligned}\dot{c}(t) &= -Dc + f(1 - c - v, c) - g(c)v \\ &\leq -(c + v)(D - f(1 - c - v, c)) \\ &\leq -(c + v)(D - D^{**}) \text{ since } c > c^{**} \\ &< -c(D - D^{**}) \text{ unless } v = 0.\end{aligned}$$

Integration of this inequality shows that on every solution not converging to  $(1, 0, 0, 0)$ , eventually  $D - g(c)$  will become positive, so the trajectory will enter the interior of  $\mathbf{B}$ .

In the interior of  $\mathbf{B}$ ,  $v(t)$  decreases strictly along solutions. By the invariance principle, the  $\omega$ -limit set of any point in  $\mathbf{X}$  is a compact, connected, nonempty invariant subset of  $\mathbf{B} \cap \{v = 0\}$ . If  $u = 0$  initially, then the solution converges to the trivial equilibrium. The maximal bounded invariant subset of the boundary consists of the two equilibria and the connecting orbit. But the nontrivial equilibrium is locally asymptotically stable, so it attracts all solutions not converging to the trivial equilibrium.

**Proposition 2** (Uniform persistence of one or both species) *If the trivial equilibrium is unstable ( $D < f(1, 0)$ ), then  $u$  is uniformly persistent in the sense of Butler, Freedman, and Waltman [6, 7], that is*

$$\exists \varepsilon > 0 : \liminf_{t \rightarrow \infty} u(t) > \varepsilon$$

for all solutions  $(s(t), u(t), c(t), v(t))_{t \geq 0}$  with  $u(0) > 0$ .

If there is coexistence in a positive equilibrium ( $D < D^*$ ), then  $u$  and  $v$  are uniformly persistent in  $\mathbf{X}$ , that is

$$\exists \varepsilon > 0 : \begin{cases} \liminf_{t \rightarrow \infty} u(t) > \varepsilon \\ \liminf_{t \rightarrow \infty} v(t) > \varepsilon \end{cases}$$

for all solutions  $(s(t), u(t), c(t), v(t))_{t \geq 0}$  with  $u(0) > 0$  and  $v(0) > 0$ .

*Proof.* We check the hypotheses of the theorem by Butler et al. [17].

1) Dissipativity. We proved that all trajectories approach the compact set

$$\{(s, u, c, v) \in \mathbf{X} \mid 0 \leq u, c, v \leq c + v \leq s + c + v = 1\}.$$

2) Weak persistence was proven earlier in this section.

3) The flow on the boundary is *isolated*, that is, the union of all  $\omega$ -limit sets of points on the invariant boundary is an isolated invariant set for the full flow. In our case, this follows from the hyperbolicity of the equilibria.

4) The flow on the boundary is *acyclic*, that is, the following oriented graph does not contain cycles.

– Vertices are the connected components of the union of all  $\omega$ -limit sets of boundary points;

– There is an edge connecting vertex  $V_1$  to vertex  $V_2$  if and only if there is a trajectory in the boundary connecting  $V_1$  to  $V_2$ , that is if for some  $\mathbf{x}$  in the boundary  $\alpha(\mathbf{x}) \subset E_1$ ,  $\omega(\mathbf{x}) \subset E_2$ .

This is satisfied, because in one case, the attractor consists of a single equilibrium, and in the other case, two equilibria are joined by a unique trajectory.

The theorem asserts that under these conditions the flow is uniformly persistent.  $\square$

*Remark.* The result on extinction of both species holds without monotonicity assumptions replacing  $f(1, 0)$  by the maximal value of  $f$ . Persistence of the first species holds without change. If  $g$  is monotonic, extinction of only the consuming species takes place for  $D^{**} \leq D < f(1, 0)$  with a suitably changed definition of  $D^{**}$ . The persistence result for both species requires monotonicity of  $f$  but not of  $g$ .

## 6 The model without toxic product effects ( $E = 0$ )

In two of the examples from the literature we gave in the introduction, the models by Kreikenbohm and Bohl for the interspecies hydrogen transfer, and the model by Wilkinson for transfer of methanol, the effect of the product on the producer is purely inhibitory. In this case the equations simplify to

$$\begin{aligned} \dot{s} &= D(1 - s) - f(s, c)u \\ \dot{u} &= u(-D + f(s, c)) \\ \dot{c} &= -Dc + f(s, c)u - g(c)v \\ \dot{v} &= v(-D + g(c)). \end{aligned}$$

Using  $u, v$  as coordinates, the flow restricted to the exponentially attracting invariant triangle

$$\{(s, u, c, v) \in \mathbf{X} \mid s + c + v = 1, s + u = 1\}$$

is generated by

$$\begin{aligned}\dot{u} &= u(-D + f(1 - u, u - v)) \\ \dot{v} &= v(-D + g(u - v)), \quad u, v \geq 0.\end{aligned}$$

This means that the long-time behaviour is governed by a system of two equations, that is, by a direct-interaction model. In particular, for linear  $f$  and  $g$ , we obtain a Volterra-type model

$$\begin{aligned}\dot{u} &= r_1 u(\kappa_1 - u + \gamma_1 v) \\ \dot{v} &= r_2 v(\kappa_2 - v + \gamma_2 u)\end{aligned}$$

with growth rates, carrying capacities, and coupling constants

$$\begin{aligned}r_1 &= f_s(1 + \alpha) & \kappa_1 &= \frac{f_s - D}{f_s(1 + \alpha)} & \gamma_1 &= \frac{\alpha}{(1 + \alpha)} \\ r_2 &= g_c & \kappa_2 &= -\frac{D}{g_c} & \gamma_2 &= 1.\end{aligned}$$

Both coupling constants are positive, as expected in a system of mutualists.  $\kappa_1$  is positive, if and only if  $D < f(1, 0) = f_s$ , that is, if and only if the producer survives in pure culture; it is a facultative mutualist.  $\kappa_2$  is always negative, indicating that the consuming species is an obligate mutualist.

The following lemma shows that for monotonic uptake functions in general the reduced equations have the positive coupling usually assumed in models for mutualists.

**Lemma** (Cooperativity) *Assume  $E = 0$ . If  $g$  is increasing and  $f_c < 0$ , then the restricted equations form a cooperative system. The resulting flow is strongly monotone (strongly order preserving) in the sense of Hirsch [13] or Matano [20] in the interior of the positive quadrant. This means that for any pair of distinct solutions  $(u_1(\cdot), v_1(\cdot))$ ,  $(u_2(\cdot), v_2(\cdot))$ , with  $u_1(0) \geq u_2(0)$  and  $v_1(0) \geq v_2(0)$ , we have  $u_1(t) > u_2(t)$  and  $v_1(t) > v_2(t)$  for all positive times.*

*If  $f_c \leq 0$  then the system is still monotone, that is, whenever  $u_1(0) \geq u_2(0)$  and  $v_1(0) \geq v_2(0)$ , we have  $u_1(t) \geq u_2(t)$  and  $v_1(t) \geq v_2(t)$  for all positive times.*

*Proof.* It is sufficient that in the linearisation matrix

$$\begin{pmatrix} * & -f_c v \\ g_c v & * \end{pmatrix}$$

the off-diagonal terms are positive if  $f_c < 0$ , and nonnegative if  $f_c \leq 0$  [13].  $\square$

**Proposition 3** (Convergence) *Assume again that  $E = 0$ ,  $f_c < 0$ , and  $g$  monotonic. The  $\omega$ -limit set of any trajectory consists of a single equilibrium. The only solutions remaining bounded for all (positive and negative) times are equilibria and connections between two of them. In particular, the stable equilibria attract the solutions through almost all initial values.*

*Proof.* By strong monotonicity, two distinct points  $(u_1, v_1)$ ,  $(u_2, v_2)$  in the  $\omega$ -limit set of a solution can never be such that simultaneously  $u_1 \geq u_2$  and  $v_1 \geq v_2$  [13]. This excludes periodic orbits and heteroclinic or homoclinic loops by the Jordan curve theorem.

Now apply the Poincaré-Bendixson theorem first to arbitrary initial values and then to points on the unstable manifolds of the equilibria to obtain the assertion.  $\square$

**Corollary** *If  $E = 0$  and both  $f$  and  $g$  are monotonic, then for any value of the parameters, there is a unique stable equilibrium attracting almost all initial values.*

*Proof.* By the results of §2, there is for each set of parameter values exactly one locally stable equilibrium.  $\square$

**Corollary** *The positive equilibrium is always a node. The slow stable manifold lies in the interior of the positive cone, the fast stable manifold in the interior of the complement. For  $D < D^*$ , the boundary equilibrium is a saddle. The unstable manifold connects the two equilibria. It is contained in the intersection of the positive cone attached at the boundary equilibrium with the negative cone attached at the positive equilibrium.*

*Proof.* The linearisation matrix at the positive equilibrium is

$$\begin{pmatrix} -(f_s - f_c)u & -f_c u \\ g_c v & -g_c v \end{pmatrix}.$$

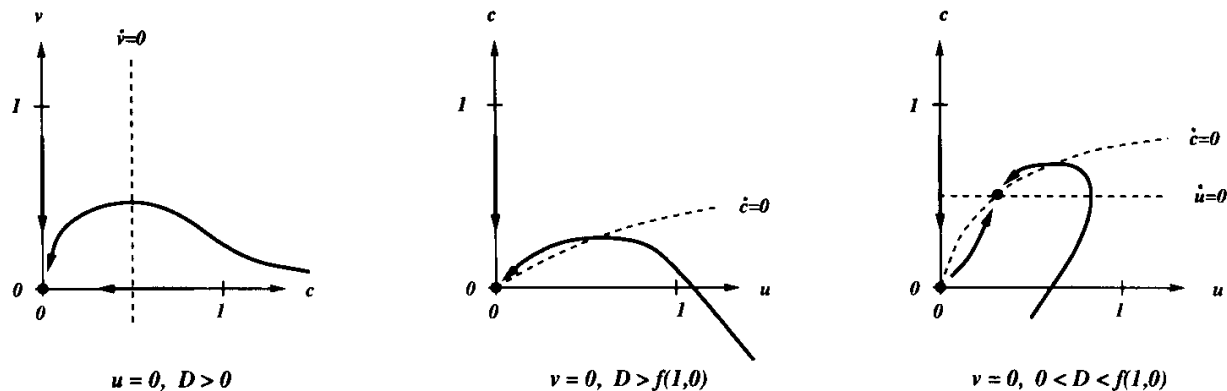
Both off-diagonal entries are strictly positive. After adding a positive multiple of the diagonal it becomes a positive matrix. Applying Perron-Frobenius theory, the largest eigenvalue is simple, real and the corresponding eigenvector lies in the positive cone. After the transformation  $v \mapsto -v$ ,  $u \mapsto u$  and reversing time, we repeat the argument to find that the eigenvector belonging to the more negative eigenvalue points into the complement of the positive cone.

The linearisation matrix in the boundary equilibrium is

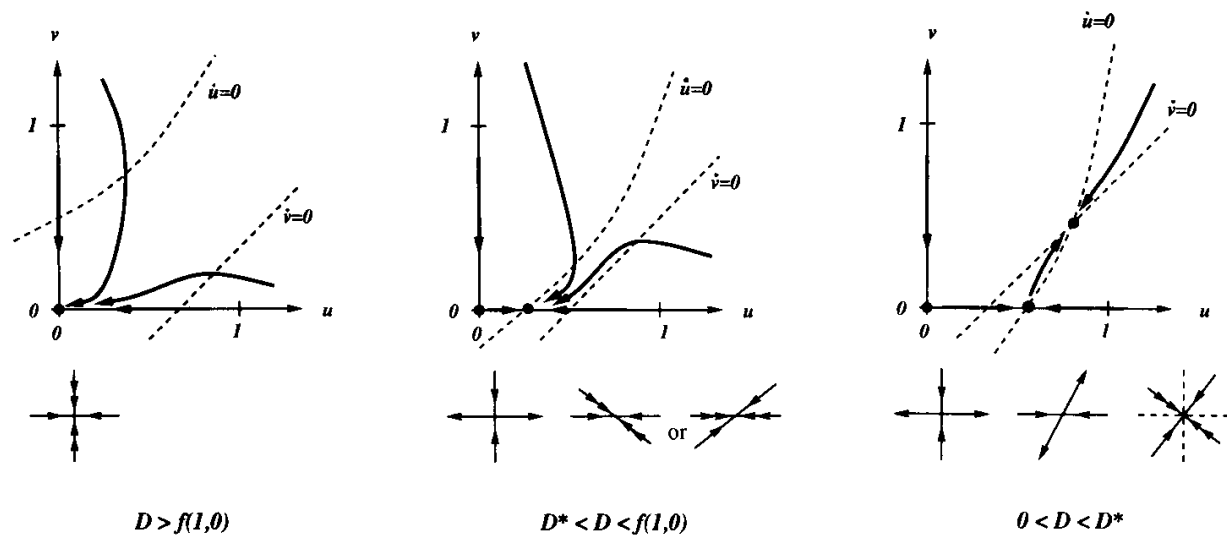
$$\begin{pmatrix} -(f_s - f_c)u & -f_c u \\ 0 & -D + g \end{pmatrix}.$$

The eigenvector belonging to the eigenvalue  $-D + g(c)$  is  $(-f_c u, (f_s - f_c)u + g - D)^t$  which is positive for  $D < D^*$ . The trajectory on the unstable manifold is initially increasing, which implies it will be increasing for all times (Hirsch [13]).  $\square$

For nonmonotone kinetics, we showed in the preceding section that the solutions will in general not behave like solutions of a cooperative system at all. With our assumption that  $f$  and  $g$  are unimodal (see §2), we see that for  $D < \min\{f(1, 0), g(1)\}$  we have exactly three equilibria, a locally attractive positive equilibrium, and the boundary and trivial equilibrium, both unstable. For  $D = 0$ , in the ‘batch’ case, both  $u$  and  $v$  are nondecreasing along trajectories, so all solutions through positive initial values have to converge to the positive equilibrium. Using invariance of the boundary curve  $\{v = 0\}$ , positive invariance of the triangle  $\{0 \leq v \leq u \leq 1\}$  and the fact that the linearisations of all equilibria for  $D = 0$  have simple real eigenvalues, we conclude that for  $D$  small enough, the phase portrait from Fig. 3 is correct.



**Fig. 3.** Dynamics on the boundary – the pure cultures. When there is no producer present, the consumer is always washed out. Without consumer, the producer is washed out if  $D \geq f(1, 0)$ . It approaches a stable positive equilibrium for  $D < f(1, 0)$



**Fig. 4.** Phase portraits and linearisations near the equilibria for the pure inhibition case ( $E = 0$ ) with monotone kinetics. Double arrows indicate the direction of the fast stable manifold

### 7 Discussion

We have used the interspecies hydrogen transfer as a model system for mutualism between two species of microorganisms. We studied associations of this kind in a chemostat as the simplest setting allowing continuous culture in a controlled environment. We wanted to understand how the interaction between the two species characterised by their nutrient requirements is influenced by the two experimentally variables dilution rate ( $D$ ) and influx substrate concentration ( $S^0$ ).

Essentially, the two species form a food chain. One species completely depends on a metabolic product of the other as a substrate. If the producing species is not influenced by the concentrations of this product, we consider the consuming species as *commensal*. Under the assumption however, that the product has some negative influence on the producer, one should expect it to benefit from the activity of the consumer, and we consider the association as *mutualistic*.

The model we discuss reflects the indirect and asymmetric character of this interaction by giving equations not only for the two species but also for the two

limiting substrates that mediate it. We compare our model for the commensal and the mutualistic case with the corresponding direct-interaction models for two species.

The simplest model equations for a mutualistic or commensal association of two species would be

$$\begin{aligned}\dot{u} &= uF(u, v) \\ \dot{v} &= vG(u, v),\end{aligned}$$

where  $F$  and  $G$  are the growth rates under the given conditions.  $F$  and  $G$  incorporate all experimental or environmental data. Standard assumption to make this a system of mutualists is that the equations form a *cooperative* system, that is, the coupling terms satisfy  $\frac{\partial F}{\partial v} > 0$ ,  $\frac{\partial G}{\partial u} > 0$ ; and for commensalists,  $\frac{\partial F}{\partial v} = 0$ . A species is called an *obligate* mutualist, if it can survive in mixed culture but not in pure culture, and *facultative* if it survives in pure culture but benefits from mixed culture. In this model, the first species is a facultative mutualist, if  $F(0, u) \geq 0$  for at least some values of  $u$ . The second species is an obligate mutualist, if always  $G(0, v) < 0$ .

This model has been discussed many times (see May [21]). Assumptions on  $F$  and  $G$  are needed to guarantee that solutions are bounded. How should one assess the mutual benefit of the two species, in particular in the case of an obligate mutualist, when pure culture is impossible? Consider for example the simplest, Volterra-type model

$$\begin{aligned}F(u, v) &= r_1(\kappa_1 - u + \gamma_1 v) \\ G(u, v) &= r_2(\kappa_2 - v + \gamma_2 u).\end{aligned}$$

The growth rates,  $r_1$ ,  $r_2$  are always positive. To make this a model for mutualism both coupling constants,  $\gamma_1$ ,  $\gamma_2$  are taken positive, for commensalism choose  $\gamma_1 = 0$ . For the first species to be a facultative, the second species an obligate mutualist, take the carrying capacities  $\kappa_1 > 0$ ,  $\kappa_2 < 0$ . None of the parameters in these models can be determined in pure cultures.

Cooperative models show very restricted dynamics. The behavior of bounded solutions is dominated by the set of equilibria. Convergence to equilibria is typically eventually monotone. Cooperative systems of two ordinary differential equations have no nontrivial periodic solutions.

We determine the coupling parameters by modeling at least partially the mechanics of the interaction. All parameters of our model are either experimentally controlled variables or characteristic of the pure cultures. This becomes an important feature, whenever one tries to use the model as a building block in a larger picture, for example a complete food web, or a spatially heterogeneous situation. We will see for our model, that parameters like ‘‘coupling constants’’ will change, when the experimental parameters are changed, and will also differ for various regions of the state space.

It turns out that in many cases the long-time behaviour is governed by a system of two differential equations as above. This happens, whenever the product has no toxic effect on the producer, in particular for the model examples given by Kreikenbohm and Bohl [17] and by Wilkinson [30]. The parameters of the reduced equations are determined by the kinetics of nutrient uptake according to the formulas

$$\begin{aligned}F(u, v) &= -D + f(S^0 - K_1 u, K_2 u - K_3 v) \\ G(u, v) &= -D + g(K_2 u - K_3 v).\end{aligned}$$

We compare the reduced equations to the standard system.

For linear uptake functions

$$f(s, c) = f_s \cdot (s - \alpha c), \quad g(c) = g_c \cdot c$$

(where  $f_s$  and  $g_c$  are positive numbers) we regain a Volterra-type model with parameters

$$\begin{aligned} r_1 &= f_s(K_1 + \alpha K_2) & \kappa_1 &= \frac{f_s S^0 - D}{f_s(K_1 + \alpha K_2)} & \gamma_1 &= \frac{\alpha K_3}{(K_1 + \alpha K_2)} \\ r_2 &= g_c K_3 & \kappa_2 &= -\frac{D}{K_3 g_c} & \gamma_2 &= \frac{K_2}{K_3} \end{aligned}$$

where  $K_1, K_2, K_3$  are the characteristic conversion constants from §1. Note that the second coupling term is positive, the first positive or zero ( $\alpha \geq 0$ ), and that the second carrying capacity  $\kappa_2$  is always negative, indicating that the consuming species is an obligate mutualist.

More generally, if  $g$  is increasing, the sign conditions for a cooperative system are satisfied, and we have a mutualistic system if  $f_c < 0$ , and a commensal one if  $f_c = 0$ . The association is facultative for the first species, obligatory for the second. We would like to argue that also for nonmonotonic  $g$ , the equations model commensalism or mutualism, respectively, although the reduced system does not have the sign structure and of a cooperative system and shows much more complicated dynamics.

In the commensal case, there is always one stable equilibrium attracting all solutions with positive initial values, provided the kinetics are unimodal in the sense of the definition in §2.

In the general case with  $f_c < 0$  consider first the species consuming the metabolic product of the other. Under the given experimental conditions, it is completely dependent on the other species. Our model reflects that, as for  $u = 0$ , complete washout of the consuming species takes place (see §4). On the other hand it is not true, unless  $g$  is monotonic, that raising the population density of the producing species always increases the growth rate of the consumer. It is true that the equilibrium, in which  $u$  is maximal, also leads to maximal densities for  $v$  and to minimal substrate concentration.

For the producing species, the association is profitable in the sense that the coupling term is positive and that equilibrium population densities in mixed culture are higher than in pure culture. It is facultative in the sense that whenever this species survives in mixed culture, it survives in pure culture as well. There are even situations in which it will reach a positive equilibrium while the other species is washed out.

The dynamical behaviour for monotone kinetics (see definition in §2) closely resembles the behaviour for the simple food chain. Successively lowering  $D$ , we see for almost all initial values first complete washout, then survival of one species in a unique stable equilibrium, then convergence to a unique equilibrium in which both species coexist. The association is obligatory for the consumer. For the producer it is optional, although its equilibrium population density and substrate utilisation is higher when the other species is present than in pure culture. This advantage becomes larger as  $D$  decreases. It is interesting to note, though, that the lowest substrate level and the highest dilution rate allowing survival of this species are not improved by the presence of the other species. In fact, close to these critical values, there is survival only in pure culture.

Experimentally, the kinetics can be made monotone by simultaneously decreasing dilution rate and influx substrate concentration in such a fashion, that their ratio

remains constant, and rescaling time accordingly. In the limit of very small dilution rates and influx concentrations, we can replace the growth functions by their linearisations at zero.

Nonmonotone kinetics of nutrient uptake lead to multiple stable equilibria and bifurcation of periodic solutions from equilibria and from separatrix loops. With these properties, the system cannot be equivalent to a cooperative system.

Among all positive equilibria, there is one in which both population densities are maximal, both substrate concentrations minimal. This equilibrium is locally attractive. The linearisation shows the positive coupling terms typical for cooperative systems. In the limit for small dilution rates, in this equilibrium, the presence of the other species becomes more advantageous for the producing species, and both substrates are completely used.

For the more general model with toxic effects, we cannot reduce to a two-species-interaction model. Still, the consuming species behaves like an obligate mutualist. The producing species survives in pure culture, sometimes even when mixed culture is not possible. We have a completely analogous bifurcation diagram. Although we suspect that for monotone kinetics still all positive solutions should converge to the unique stable equilibrium, we were not able to show this analytically. We obtain critical values of the dilution rate for extinction and persistence. We also show bifurcation of periodic solutions from equilibria near Bogdanov-Taken singularities for nonmonotone kinetics.

In the same model, changing only the parameters  $S^0$  and  $D$  proportionally, we see a standard cooperative system if these parameters are small. If these parameters are large, the association still has many features of a mutualistic system. One species behaves as an obligate mutualist, completely dependent on the other, but not always benefitting from growth of the other species. The other species always benefits (in the sense that its growth rate is increased by the presence of the other) but survives in pure culture, does not depend on the other species. There is one maximal equilibrium, near which the system more closely resembles a traditional mutualistic system. This equilibrium is stable. For  $D$  small enough, it is the only stable equilibrium, and we expect it to dominate the dynamics. In general, however, our system shows complicated dynamics that can never occur in a cooperative system.

*Acknowledgement.* I would like to thank Willi Jäger for suggesting the problem to me, and for his attention and support during my years in Heidelberg.

## References

1. Arnol'd, V. I.: Lectures on bifurcation in versal families. *Russ. Math. Surv.* **27**, 54–123 (1972)
2. Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., Wolfe, R. S.: Methanogens: Reevaluation of a Unique Biological Group. *Microbiol. Rev.* **43**, 260–296 (1979)
3. Bogdanov, R. I.: Versal Deformations of a Singularity of a Vector Field on the Plane in the Case of Zero Eigenvalues. *Sel. Math. Sov.* **1**, 389–421 (1981)
4. Bryant, M. P., Wolin, E. A., Wolin, M. J., Wolfe, R. S.: Methanobacillus omelianskii, a Symbiotic Association of Two Species of Bacteria. *Arch. Mikrobiol.* **59**, 20–31 (1967)
5. Burchard, A.: Ein Chemostat-Modell für den Abbau eines komplexen Substrats durch eine symbiotische Assoziation zweier Spezies. Diplomarbeit, Universität Heidelberg 1989
6. Butler, G. J., Waltman, P.: Persistence in Dynamical Systems. (Preprint)



7. Butler, G. J., Freedman, H. I., Waltman, P.: Uniformly Persistent Systems. *Proc. Am. Math. Soc.* **96**, 425–430 (1986)
8. Chow, S.-N., Hale, J.: *Methods of Bifurcation Theory*. Berlin Heidelberg New York: Springer 1982
9. Fiedler, B.: An Index for Global Hopf Bifurcation in Parabolic Systems. *J. Reine Angew. Math.* **359**, 1–36 (1985)
10. Fiedler, B.: Global Hopf Bifurcation of Two Parameter Flows. *Arch. Ration. Mech. Anal.* **94**, 59–81 (1986)
11. Gottschal, J. C., Dijkhuizen, L.: The Place of the Continuous Culture in Ecological Research. In: Wimpenny, J. W. T. (ed.) *Handbook of Laboratory Model Systems for Microbial Ecosystems*, vol. 1. Boca Raton, FL: CRC Press 1988
12. Guckenheimer, J., Holmes, P.: *Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields*. (Appl. Math. Sci., vol. 42) Berlin Heidelberg New York: Springer 1983
13. Hirsch, M. W.: Stability and convergence in strongly monotone dynamical systems. *J. Reine Angew. Math.* **383**, 1–53 (1988)
14. Hungate, R. E.: Hydrogen as an Intermediate in the Rumen Fermentation. *Arch. Mikrobiol.* **59**, 158–164 (1967)
15. Hungate, R.E.: A Roll Tube Method for Cultivation of Strict Anaerobes. In: Norris, R., Ribbons, D. W. (eds.) *Methods in Microbiology*, vol. 3B. New York: Academic Press 1969
16. Ianotti, E. L., Kafkewitz, D., Wolin, M. J., Wolfe, R. S.: Glucose fermentation products of *ruminococcus albus* grown in continuous culture with *vibrio succinogenes*. *J. Bacteriol.* **114**, 1231–1240 (1973)
17. Kreikenbohm, R., Bohl, E.: A Mathematical Model of Syntrophic Cocultures in the Chemostat. *FEMS Microbiol. Ecol.* **38**, 131–140 (1986)
18. Mah, R. A., Smith, M. R.: The Methanogenic Bacteria. In: *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria*. Berlin Heidelberg New York: Springer 1981
19. Marsden, J. E., McCracken M.: *The Hopf Bifurcation and its Applications*. Berlin Heidelberg New York: Springer 1976
20. Matano, H.: Strongly order-preserving local semidynamical systems - theory and applications. In: Brezis, H. et al. (eds.) *Semigroups, theory and applications*, vol. I. Trieste 1984. (Pitman Res. Notes Math. Ser., vol. 141, pp. 178–185) Harlow: Longman 1986
21. May, R. M. (ed.): *Theoretical Ecology*, 2nd edition. Sunderland, MA Sinauer: 1981
22. McInerney, M. J., Bryant, M. P., Pfennig, N.: Anaerobic Bacterium that Degrades Fatty Acids in Syntrophic Association with Methanogens. *Arch. Microbiol.* **122**, 129–135 (1979)
23. Mountfort, D. O., Bryant, M. P.: Isolation and Characterization of an Anaerobic Benzoate-Degrading Bacterium from Sewage Sludge. *Arch. Microbiol.* **133**, 249–256 (1982)
24. Orenski, S. W.: Intermicrobial Symbiosis. In: Henry, S. M. (eds.) *Symbiosis*, vol. 1. New York London: Academic Press 1966
25. Otto, R., Hugenholz, J., Konings, W. N., Veldkamp, H.: Increase in molecular growth of *streptococcus cremoris* for lactose as a consequence of lactate consumption by *pseudomonas stutzeri* in mixed culture. *FEMS Microbiol. Lett.* **9**, 85 (1980)
26. Pollock, M. R.: A case of bacterial symbiosis based on the combined growth stimulating and growth inhibiting properties of long-chain fatty acids. *J. Gen. Microbiol.* **2**, xxiii (1948)
27. Powell, G. E.: Equalisation of Specific Growth Rates for Syntrophic Associations in Batch Culture. *J. Chem. Tech. Biotechnol.* **34**, 97–100 (1984)
28. Takens, F.: Singularities of vector fields. *Publ. Math., Inst. Hautes Étud. Sci.* **43**, 47–100 (1974)
29. Traore, A. S., Fardeau, M. L., LeGall, J., Belaich, J. P.: Energetics of growth of a defined mixed culture of *desulfovibrio vulgaris* and *methanosarcina barkeri*: Interspecies hydrogen transfer in batch and continuous cultures. *Appl. Environ. Microbiol.* **46**, 1152 (1983)
30. Wilkinson, T. G., Topiwala, H. H., Hamer, G.: Interactions in a mixed bacterial population growing on methane in continuous culture. *Biotechnol. Bioeng.* **16**, 41 (1974)
31. Vanderbauwhede, A.: Center manifolds, normal forms and elementary bifurcations. *Dyn. Rep.* **2**, 89–169 (1989)
32. Zeikus, J. G.: The Biology of Methanogenic Bacteria. *Bacteriol. Rev.* **41**, 514–541 (1977)