Standing Wave Patterns in Models for Microbial Growth

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Abstract: A kinetic model for microbial growth dynamics taking into account that cells can exist in two phases, fast growing and slow growing, is extended to spatially nonuniform conditions. The resulting reaction-diffusion system with unequal diffusion coefficients exhibits standing wave patterns as can be predicted via conditions concerning the kinetic equations. These solutions come from a Hopf bifurcation of Turing type. In the whole range of parameters there are no time-periodic solutions without diffusion.

1 Introduction

A modified Monod model for microbial growth under nutrient limitation was already presented in detail in [7]. Therein a system of three ordinary differential equations was suggested, modeling the nutrient concentration and two cell populations. Due to a change of metabolism, cells can switch from a quiescent to an active state. A transient oscillation of cell concentration and nutrient to an asymptotically stable steady state, that had been observed experimentally (see for example [2]), could be explained with the help of this metabolic change.

This paper concentrates on spatially nonconstant distributions. In Section 2 the main ideas of analyzing the stabilization effects of submatrices of the Jacobian at the stable equilibrium point are presented. With this tool at hand, the role of the different terms of the mathematical models can be identified to be responsible for a certain qualitative behavior.

In order to illustrate the theoretic approach a model for microbial growth is given in Section 3. The system of equations is derived from a chemostat model and additionally considers a diffusion term for the cell movement. Detailed knowledge of the kinetic system forms the basis for the investigation of nonconstant steady and periodic solutions. Section 4 treats a simpler model that is already sufficient to explain transient oscillations to a constant steady state that allows both cell cultures to coexist in the same vessel. But it is not sufficient to explain any sustained spatial patterns bifurcating from constant solutions. Therefore it is necessary to have a growth rate function for the cells of the second phase. The analysis on this model is carried out in Section 5.

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Finally the predicted results are verified numerically for a given set of parameters and functions fulfilling all the required conditions.

2 Wave Bifurcation

Consider a reaction-diffusion system of the form

$$\begin{array}{lll} u_t = D\Delta u + F(u,\Lambda) & \text{on} & \Omega \times \mathbb{R}^+ \\ u(0,x) = u_0(x) & \text{on} & \Omega \times 0 \\ \partial_n u = 0 & \text{on} & \partial\Omega \times \mathbb{R}^+ \end{array} \tag{1}$$

on a domain $\Omega \subset \mathbb{R}^n$ with no-flux boundary conditions, where the solution u is a vector valued function, D is a diagonal square matrix of diffusion coefficients and F describes the kinetic of the system. This functional F is not explicitely time dependent but contains nonlinearities and depends on a set of real parameters Λ.
This paper investigates the case with symmetric diagonal principal part, where the coupling takes place in the reaction terms. See for instance [1] for an example of a periodic nonconstant solution of a two variable system in the case of strongly coupled quasilinear reaction-diffusion systems.
A wave bifurcation is a supercritical Hopf bifurcation from a stable steady constant solution to a stable periodic and nonconstant solution. This can be regarded as a standing wave solution that is periodic in space and time. The constant solution is an equilibrium point of the kinetic system. The aim is to destabilize this solution by diffusion coefficients, as in the case of the classical *Turing Instability*, where a single eigenvalue becomes positive and the bifurcating solution is a nonconstant steady state. If the real part of a single pair of complex eigenvalues of the linearization crosses the imaginary axis, this results in a nonconstant periodic solution.
It is not possible to get a wave bifurcation for a two variable system (see TURING [13], and therein the cases e) and f): e) *Oscillatory case with a finite wave-length and* f) *Oscillatory case with extreme short wave-length,* [...] *possibilities* [that] *can only occur with three or more morphogens.* [...] *no attempt was made to develop formulae for these.*).
The attempt is now to give formal conditions for the dynamics that make it possible to arrange a destabilization of a constant solution for a three variable system. The main idea is to compare the Jacobian A of the kinetic system F to the linearization of SYSTEM (1) at a constant solution, denoted by $\tilde{A}$.
At first some notations are introduced: The tilde $\sim$ always denotes the PDE system and the index m indicates the respect to the appropriate mode via the eigenvalue μ_m of the negative Laplace operator with Neumann boundary conditions. If Ω is the unit interval, $-\Delta\xi_m = \mu_m\xi_m$ results in $\xi_m = cos(\pi m x)$ the eigenfunction for the eigenvalue $\mu_m = (\pi m)^2, m \in \mathbb{N}_0$. Claiming that all eigenfunctions of $\tilde{A}$ have the form $(c_1, c_2, c_3)^t\xi_m$, this yields a three dimensional subspace of functions which is invariant for $\tilde{A}$. By Fourier analysis and arguing componentwise, the eigenfunctions of $\tilde{A}$ provide a complete set for vector functions. $\tilde{A}(\mu_m)$ denotes the restriction of $\tilde{A}$ to the three dimensional subspace of

the mth spatial mode. The diffusion coefficients therefore appear in the Jacobian as the product $d_i\mu_m,\ i = 1,2,3$.

$$\tilde{A}(\mu_m) = A - D(\mu_m) = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} - \begin{pmatrix} d_1 & 0 & 0 \\ 0 & d_2 & 0 \\ 0 & 0 & d_3 \end{pmatrix} \mu_m$$

The trace of a submatrix of A consisting of the i_1 and i_2 column and row will be denoted as $\mathrm{tr}(A_{i_1 i_2})$. The minors with the same two columns and rows will be abbreviated with $|A_{i_1 i_2}|$.

Now recall that the eigenvalues of a system of three equations are the roots of the characteristic polynomial

$$\chi_A(\lambda) = \lambda^3 - \mathrm{tr}A\ \lambda^2 + \sum_{1\le i<j\le 3} |Aij|\ \lambda - \mathrm{det}A = 0.$$

The number k of eigenvalues in the right half plane for a system of three equations is computed with Routh-Hurwitz' Theorem (see for instance Gantmacher [5]) and yields

$$\begin{aligned} k = V\left(1, \Delta_1, \frac{\Delta_2}{\Delta_1}, \frac{\Delta_3}{\Delta_2}\right) &= V(1, \Delta_1, \Delta_3) + V(1, \Delta_2) \\ &= V(1, \Delta_1) + V(\Delta_1, \Delta_3) + V(1, \Delta_2) \end{aligned}$$

where V counts the number of sign changes between adjacent members in the sequence. Δ_i are the successive Hurwitz determinants to be computed using the coefficients $(-1)^i\sigma_i$, $i = 1, 2, 3$ of the symmetric functions.

$$\begin{array}{lclcl} \Delta_1 & = & -\mathrm{tr}A & = & -\sigma_1 \\ \Delta_2 & = & -\mathrm{tr}A(\sum |A_{ij}|) + \mathrm{det}A & = & -\sigma_1\sigma_2 + \sigma_3 \\ \Delta_3 & = & -\Delta_2\ \mathrm{det}A & = & -(-\sigma_1\sigma_2 + \sigma_3)\sigma_3 \end{array}$$

The number k is zero and consequently the equilibrium point is asymptotically stable, if and only if all Δ_i are positive. For reaction-diffusion systems, the number of eigenvalues in the right half plane is the sum $\tilde{k} := \sum_{m=0}^{\infty} k_m$, where the nonnegative number k_m belongs to the mth spatial mode. Therefore one has to compute the Hurwitz determinants of all $\tilde{A}(\mu_m)$. The sum $\tilde{k}$ is zero, if and only if the sign conditions are now fulfilled for all m, that is $\tilde{\Delta}_i(\mu_m) > 0,\ i = 1, 2, 3$.

Destabilization of the constant solution by diffusion is observed, if the number k is zero for the matrix A and the sum $\tilde{k}$ is positive. Perturbations of the constant solution in the space of constant functions then always settle again to this constant solution; but inhomogeneous perturbations may fold up to nonconstant solutions. This situation occurs if at least one of the $\tilde{\Delta}_i(\mu_m)$ becomes negative.

A stable equilibrium for the reaction system has $-\mathrm{tr}A > 0$ or in other terms $V(1, \Delta_1) = 0$. Adding diffusive terms to the trace will only decrease the trace and therefore increase $-\mathrm{tr}\tilde{A}(\mu_m)$. It yields $-\mathrm{tr}\tilde{A}(\mu_m) > -\mathrm{tr}A > 0$, and $-\mathrm{tr}\tilde{A}(\mu_m)$ is always positive.

In the case of *Turing Instability* a single eigenvalue crosses for a single m. Therefore $\det\tilde{A}(\mu_m)$ has to change sign, and a steady nonconstant solution is achieved as described for various biological examples in the book of MURRAY [10].
It will be shown that in the case of *Wave Bifurcation* two complex conjugate eigenvalues cross the imaginary axis, and again this can happen for a single m. If $\tilde{\Delta}_i(\mu_l) > 0$ for all $l \neq m,\ i = 1, 2, 3,\ \tilde{\Delta}_1(\mu_m) > 0,\ \tilde{\Delta}_3(\mu_m) > 0$, but $\tilde{\Delta}_2(\mu_m) < 0$ for a single m, the overall sum $\tilde{k} = k_m$ increases by two. This situation can be adjusted with the diffusion coefficients as bifurcation parameters.
The case of $N = 3$ variables is the smallest number of equations that can have a bifurcation to a spatially nonconstant and temporally periodic solution by unequal diffusion coefficients.
This is possible because of the surface $\sigma_1\sigma_2 = \sigma_3,\ \sigma_2 > 0$. The trace of the linearization is negative, the determinant indicates an even number of eigenvalues in the right half plane, and $\tilde{k}$ increases when crossing through this surface, while the determinant remains negative (see FIGURE 2). In two variable systems this is not possible, since for the regular as for the singular perturbation, that is $d_1 \to \infty$ or $d_2^{-1} \to \infty$, the Hopf bifurcation can only be realized when the trace becomes positive. But this means a Hopf bifurcation already for the kinetic system and yields a constant time-periodic solution. Note that a constant but time-periodic solution has Neumann boundary conditions, a difficulty that does not occur in the case of Dirichlet boundary conditions that are not discussed here.

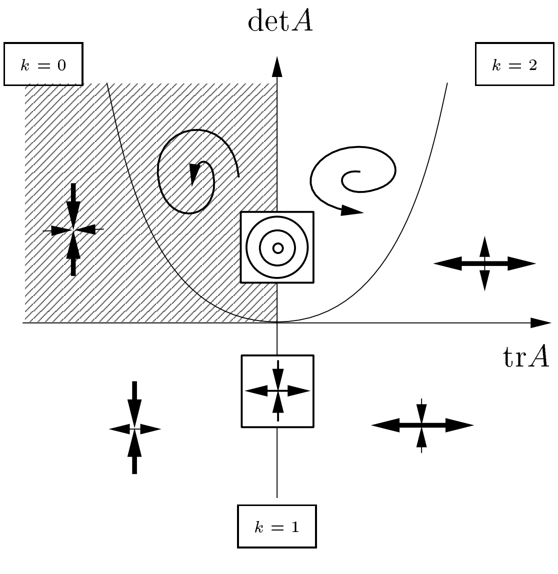

Figure 1: For a two variable system a sign change of the trace results in an increase of k by 2.

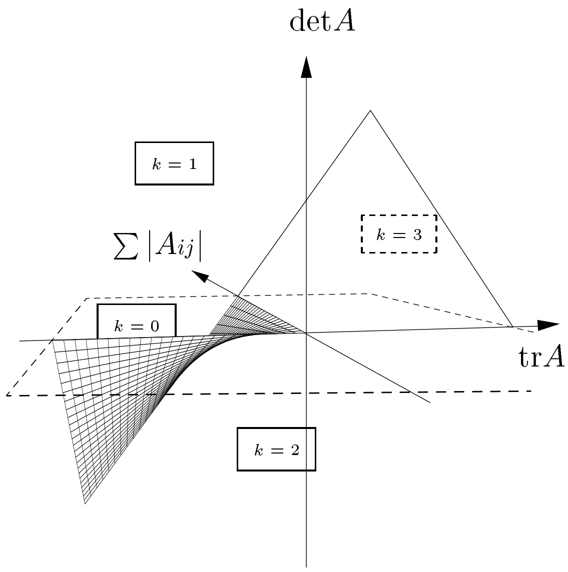

Figure 2: For a three variable system the surface $\sigma_1\sigma_2 = \sigma_3,\ \sigma_2 > 0$, has to be crossed to increase k by 2.

To illustrate the formal computations of Hurwitz determinants, the figures above give an impression, where the regions of stable nodes are ($k = 0$, shaded areas), and which lines or surfaces separate these regions for the various numbers of k in two and three variable systems. Qualitatively the pictures do not change when considering $\tilde{A}(\mu_m)$ and $\tilde{k}_m$ instead of A and k, respectively.

2.1 Nonconstant Steady States

In the sense of *activator-inhibitor* systems, the activator is the variable, that causes the instability. Two variable reaction-diffusion systems with activator-inhibitor kinetic are widely studied (see for example [4]). To compare them to systems showing a wave bifurcation, the necessary conditions on the kinetic equations are:

Conditions for Turing Bifurcation:

(c1) $-\text{tr}A > 0$

(c2) $\det A > 0$

(c3) $a_{22} > 0$

Then an appropriate diffusion coefficient d_1 can make the term $a_{11}d_2 + a_{22}d_1$ positive, although the trace $a_{11} + a_{22}$ is negative, and thereby change the sign of the determinant in the presence of spatial inhomogeneities. That is often abbreviated in the rule:

$$\frac{d_1}{d_2} > \frac{-a_{11}}{a_{22}} > 1 \qquad \Rightarrow \qquad \text{The inhibitor has to diffuse faster.}$$

Remark 1 (c1) *and* (c2) *guarantee a stable equilibrium,* (c3) *demands the presence of an activator, that is in the case of two variable systems just a positive diagonal entry in the linearization of the second equation, the activator equation.*
In a generalization for N-variable systems the N^{th} condition for the stability of the constant solution is $(-1)^N \det A > 0$*. M denotes the submatrix of linearized kinetic equations with smaller (or zero) diffusion coefficients. A generalized Turing instability is archieved for* $(-1)^{(N-1)}\det M < 0$*, with the submatrix M playing the role of the activator.*

2.2 Nonconstant Periodic Solutions

For a wave bifurcation, when $\tilde{k}$ jumps from 0 to 2, $\tilde{k}$ is even and one can prove, that then $(-1)^N \det\tilde{A}(\mu_m)$ is positive.
The idea behind the wave bifurcation uses Orlando's formula (see again [5])

$$\Delta_{N-1} = (-1)^{\frac{N(N-1)}{2}} \prod_{i<k}^{1\ldots N} (\lambda_i + \lambda_k)$$

from which it follows that $\Delta_{N-1} = 0$ if and only if the sum of at least one pair of roots of the polynomial is zero. In particular this is true for a conjugate pair of pure imaginary roots. Together with the Routh-Hurwitz Theorem the case of a single pair of pure imaginary roots and all other roots with negative real part can be characterized by:

(a) $\Delta_i > 0$ for $i = {\scriptstyle 1,\ldots,N-2}$

(b) $\Delta_{N-1} = 0$

(c) $(-1)^N \det A > 0$

The last inequality is needed to stay away from zero as a (multiple) root.
To derive conditions for the situation, that the kinetic system has a stable constant solution, that is destabilized in the reaction-diffusion system, it only makes sense to look at a system with three variables. So for the Hopf bifurcation the second Hurwitz determinant has to be investigated. Comparing $\tilde{\Delta}_2(\mu_m d_1, d_2, d_3)$ with Δ_2 yields

$$
\begin{aligned}
\tilde{\Delta}_2(\mu_m, d_1, d_2, d_3) = \ & [\ 2d_1d_2d_3 + (d_1)^2(d_2+d_3) + (d_2)^2(d_1+d_3) + (d_3)^2(d_1+d_2)\ ]\ {\mu_m}^3 \\
& -[\ \mathrm{tr}(A_{23})(d_1)^2 + \mathrm{tr}(A_{13})(d_2)^2 + \mathrm{tr}(A_{12})(d_3)^2 + \\
& \quad 2\mathrm{tr}A(d_1d_2 + d_1d_3 + d_2d_3)\ ]\ {\mu_m}^2 \\
& +[\ (|A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A)d_1 + \\
& \quad (|A_{12}| + |A_{23}| + \mathrm{tr}(A_{13})\mathrm{tr}A)d_2 + \\
& \quad (|A_{13}| + |A_{23}| + \mathrm{tr}(A_{12})\mathrm{tr}A)d_3\ ]\ \mu_m \\
& +\ \Delta_2\ .
\end{aligned}
$$

Now consider some positive d_1 and a singular limit with $d_2 \to 0$ and $d_3 \to 0$. The formal limit

$$
\begin{aligned}
\lim_{\substack{d_2\to 0\\ d_3\to 0}} \tilde{\Delta}_2(\mu_m, d_1, d_2, d_3) &= \tilde{\Delta}_2(\mu_m, d_1, 0, 0) \\
&= -\mathrm{tr}(A_{23})(d_1\mu_m)^2 + (|A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A)d_1\mu_m + \Delta_2
\end{aligned}
\tag{2}
$$

gives a parabola $\tilde{\Delta}_2(\mu_m, d_1, 0, 0) =: \tilde{\Delta}_2(d_1\mu_m)$ in d_1. The following conditions for a change in sign of $\tilde{\Delta}_2(d_1\mu_m)$ by the diffusion coefficient d_1 and the spatial mode μ_m imply wave bifurcation.

Conditions for Wave Bifurcation:

(C1) $-\mathrm{tr}A > 0$

(C2) $\Delta_2 = -\mathrm{tr}A(\sum |A_{ij}|) + \det A > 0$

(C3) $-\det A > 0$

(C4) $-\mathrm{tr}(A_{23}) > 0$

(C5) $-(|A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A) > 0$

(C6) $(|A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A)^2 + 4\mathrm{tr}(A_{23})\Delta_2 > 0$

Remark 2 (C1) – (C3) *guarantee a stable constant equilibrium of the kinetic system. The normed parabola to be discussed is*

$$\frac{1}{-\mathrm{tr}(A_{23})}\tilde{\Delta}_2(d_1\mu_m) = (d_1\mu_m)^2 - \frac{(|A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A)}{\mathrm{tr}(A_{23})} d_1\mu_m - \frac{\Delta_2}{\mathrm{tr}(A_{23})}$$

that is the normed second Hurwitz determinant depending on the diffusion coefficient d_1 *and the spatial mode* μ_m. (C4) *is needed for the right opening of the parabola,* (C5) *is needed for positive real parts of the roots and* (C6) *is sufficient for the existence of two real roots* $\nu^{\pm} > 0$. *Now the second Hurwitz determinant becomes negative for* $d_1\mu_m \in (\nu^-, \nu^+)$ *and this results in complex eigenvalues for the Jacobian* $\tilde{A}$. *Note that all these conditions only concern the kinetic system.*

Theorem 1 *Consider* SYSTEM (3) *of a parabolic and two ordinary differential equations that has an asymptotically stable constant equilibrium* E^* *(i.e.* (C1) – (C3) *are satisfied).*

$$\begin{array}{rcl} u_{1_t} &=& B(u_1, d_1) + g_1(u_1, u_2, u_3) \\ u_{2_t} &=& g_2(u_1, u_2, u_3) \\ u_{3_t} &=& g_3(u_1, u_2, u_3) \end{array} \qquad \text{in } \Omega \times (0, \mathrm{T}^+) \qquad (3)$$

and appropriate boundary conditions such that the spectrum of $-B(u_1, d_1)$ *is nonnegative, real and discrete. The linearized kinetic system at the constant solution fulfills* (C4) – (C6). *Now*

$$\begin{array}{rcl} \tilde{\Delta}_2(d_1^*\mu_m) &=& 0 \quad \text{for } m \\ \tilde{\Delta}_2(d_1^*\mu_l) &>& 0 \quad \text{for } l \neq m \end{array}$$

for a single $m \in \mathbb{N}$, *where* μ_m *denotes the* m^{th} *eigenvalue of* $-B(u_1, d_1)$. *Then*

(i) $\{\pm i\omega\}$ *are simple eigenvalues of* $\tilde{A}$, *where* $\omega > 0$,

(ii) *there are no eigenvalues of the form* $ik\omega$ *for* $k \in \mathbb{Z} \setminus \{\pm 1\}$ *and*

(iii) $\partial_{d_1}\mathrm{Re}\rho(d_1^*) \neq 0$, *where* $\rho(d_1)$ *is the unique continuation of the eigenvalue of* $\tilde{A}$ *for* d_1 *in a neighborhood of the critical* d_1^* *satisfying* $\rho(d_1^*) = i\omega$.

Then the SYSTEM (3) *has a unique one-parameter family of noncritical nonconstant periodic orbits in an appropriate neighborhood; precisely for* $u := (u_1, u_2, u_3)$, *the minimal period* T *and the bifurcation parameter* d_1 *it yields*

$$(u(\cdot), T(\cdot), d_1(\cdot)) \ \in \ C^\infty((-\varepsilon, \varepsilon), V \times \mathbb{R} \times \mathbb{R})$$

satisfying

$$(u(0), T(0), d_1(0)) = (E^*, \frac{2\pi}{\omega}, d_1^*)$$

such that

$$\gamma(s) := \gamma(u(s))$$

is a noncritical nonconstant periodic orbit of SYSTEM (3) *of period* $T(s)$ *passing through* $u(s) \in V$ *for* $0 < s < \varepsilon$.

Remark 3 *Via cyclic changes of the indices* $i = 1, 2, 3$ *in* (C4) – (C6), THEOREM 1 *can be formulated for a spatial operator in the second or third equation.*

The following figure illustrates the picking of a single mode that becomes unstable.

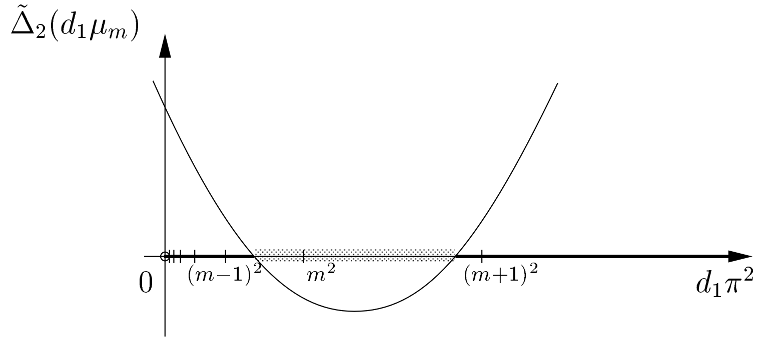

Figure 3: The parabola $\tilde{\Delta}_2(d_1\mu_m)$ selects a single mode of oscillating solutions, when the kinetic system is regularly perturbed with a diffusion operator in a single equation (here for the Laplace operator on a one dimensional domain).

Proof (of THEOREM 1*):* The local existence of a bifurcating periodic solution follows from CRANDALL, RABINOWITZ [3] in the case that (i) – (iii) can be satisfied. These three conditions are a consequence of the conditions imposed on the kinetic equations, i.e. (C1) – (C6), and those were derived from conditions (a), (b) and (c).
It remains to prove that $-\det\tilde{A}(\mu_m, d_1, d_2, d_3) > 0$ (see FIGURE 2), which guarantees an even number of eigenvalues with positive real part. Consider the following limit:

$$\lim_{\substack{d_2 \to 0 \\ d_3 \to 0}} \left(-\det\tilde{A}(\mu_m, d_1, d_2, d_3)\right) =: -\det\tilde{A}(d_1\mu_m) = |A_{23}| d_1\mu_m - \det A \tag{4}$$

If $|A_{23}|$ is positive, then $-\det\tilde{A}(d_1\mu_m) > -\det A > 0$ for all d_1 and all m, so that there is no change in sign of the determinant. The above conditions already imply that $|A_{23}|$ is positive, and this is proved in the following lemma.

Lemma 1 *Let* A *be such that* (C1) – (C5) *are fulfilled. Then* $|A_{23}|$ *is positive.*

Proof:

$$\begin{aligned} (\mathrm{C5}) \;\Rightarrow\;& |A_{12}| + |A_{13}| + \mathrm{tr}(A_{23})\mathrm{tr}A < 0 \\ \Leftrightarrow\;& |A_{12}| + |A_{13}| < -\mathrm{tr}(A_{23})\mathrm{tr}A \overset{(\mathrm{C1})(\mathrm{C4})}{<} 0 \\ \Rightarrow\;& |A_{12}| + |A_{13}| < 0 \end{aligned}$$

Assume $|A_{23}| \leq 0$, then

$$0 \; < \; \mathrm{tr}A\Big(|A_{12}| + |A_{13}| + |A_{23}|\Big) \overset{(\mathrm{C2})}{<} \det A \overset{(\mathrm{C3})}{<} 0$$

which is a contradiction. □

For the wave bifurcation $|A_{23}|$ has to be positive. But since EQUATION (2) might have no positive roots this is not sufficient. A simple computation shows that existence of positive roots can only be achieved if (C5) and (C6) are satisfied. □

Nevertheless the positivity of $|A_{23}|$ can be used as a first check whether wave bifurcation is possible or not. A submatrix with only positive Hurwitz determinants (that means for a 2×2 matrix a negative trace and positive determinant) will be called stabilizing. There are two different cases that can be distinguished for SYSTEM (3) at an asymptotically stable constant solution:

- $|A_{23}| < 0$ is the necessary (and sufficient) condition to arrange a Turing bifurcation. This submatrix is therefore not stabilizing.
- $|A_{23}| > 0$ is the necessary (but not sufficient) condition to arrange a wave bifurcation. (C4) and (C5) imply $\mathrm{tr}(A_{23}) < 0$ and $|A_{23}| > 0$. This submatrix is stabilizing.

Remark 4 *A simultaneous change of sign of* $\det\tilde{A}(d_1\mu_m)$ *and* $\tilde{\Delta}_2(d_1\mu_m)$ *would also lead to a Turing instability (see* [8], $k = 1$*). But this cannot happen in the limiting case where both* d_2 *and* d_3 *are zero, because* $|A_{23}|$ *has to have opposite signs for the simultaneous change. The case* $|A_{23}| = 0$ *does not make sense here.*

The results of the computations on the stability at the equilibrium point can now be summarized.

Remark 5 *At a stable constant solution the sum* $\sum |A_{ij}|$ *is positive. For wave bifurcation at least one* $|A_{jk}|$ *has to be negative to satisfy* (C5)*. The spatial operator occurs in the* j^{th} *or* k^{th} *equation. The purely kinetic submatrix is stabilizing.*

2.3 Estimation of Mode Selection

Further investigations of the wave bifurcation concern the biggest mode M that can be isolated. For one dimensional domains Ω and the Laplace operator, this wavenumber is estimated by $\nu^{\pm}$, the roots of $\tilde{\Delta}_2(d_1\mu_m)$ (see FIGURE 3). For all integers m less than M, a bifurcation to a wave solution with the appropriate wavenumber can be arranged either for shrinking or growing d_1. But for wavenumbers greater than M, at least two modes are within the unstable area. This doesn't mean that there is no such solution, but it strongly depends on the initial data, which solution will survive.

Lemma 2 *The biggest wavenumber M for which only a single mode is destabilized in a one space dimensional system can be estimated by* $\mu^- := \dfrac{2\nu^-}{\nu^+ - \nu^-}$ *with*

$$\mu^- - 1 < M < \mu^- + \frac{1}{2}$$

where $\nu^{\pm}$ are the roots of the parabola $\tilde{\Delta}_2(d_1\mu_m)$ of THEOREM 1.

Proof: Fix the left root ν^- at the M^{th} mode with the scaling factor $d_1\pi^2$.

$$d_1\pi^2 M^2 = \nu^- \tag{5}$$

Maximizing the scaling factor gives the following inequalities. EQUATION (6) is for not to get a second mode into the unstable region, EQUATION (7) is to be greater than the previous spacing between two eigenvalues.

$$\nu^+ - \nu^- \; < \; d_1\pi^2((M+1)^2 - M^2) \; = \; d_1\pi^2(2M+1) \tag{6}$$

$$d_1\pi^2(M^2 - (M-1)^2) \; = \; d_1\pi^2(2M-1) \; < \; \nu^+ - \nu^- \tag{7}$$

Estimation from above is done with EQUATION (5) and EQUATION (6).

$$\frac{2M^2}{2\nu^-} = \frac{1}{d_1\pi^2} < \frac{2M+1}{\nu^+ - \nu^-}$$

$$\Rightarrow \quad \frac{2M^2}{2M+1} = M - \frac{M}{2M+1} < \frac{2\nu^-}{\nu^+ - \nu^-} = \mu^-$$

$$\Rightarrow \quad M - \frac{1}{2} < M - \frac{M}{2M+1} < \mu^- \quad \Rightarrow \quad M < \mu^- + \frac{1}{2}$$

For the estimation from below EQUATION (5) and EQUATION (7) are used.

$$\frac{2M-1}{\nu^+ - \nu^-} < \frac{1}{d_1\pi^2} = \frac{2M^2}{2\nu^-}$$

$$\Rightarrow \mu^- < \frac{2M^2}{2M-1} = \frac{M(2M-1)+M}{2M-1} = M + \frac{M}{2M-1} \leq M+1 \;\Rightarrow\; \mu^- - 1 < M$$

There are at most two integers within the bounds. It has to be checked if $\tilde{\Delta}_2$ always evaluates negative for both modes (then take the smaller one), or only for one of them. □

Once the maximal M is found, the critical $d_{1_{M_r}}$, for which there is bifurcation to the standing wave solution for shrinking $d_1 < d_{1_{M_r}}$, can be calculated easily. In the same way a critical $d_{1_{M_l}}$ is calculated for $M-1$, and there is a bifurcation to a time-periodic solution for growing $d_1 > d_{1_{M_l}}$. The two possibilities resemble the situation to either touch the square number with the right or left wing of the parabola. This yields the following sequence

$$\cdots\ d_{1_{M_l}} < d_{1_{M_r}} < d_{1_{(M-1)_l}} < d_{1_{(M-1)_r}}\ \cdots$$

and there is no mode destabilized within the open interval $(d_{1_{M_r}}, d_{1_{(M-1)_l}})$. So the mode destabilization is well separated up to the mode with the maximal number that can be estimated with Lemma 2.

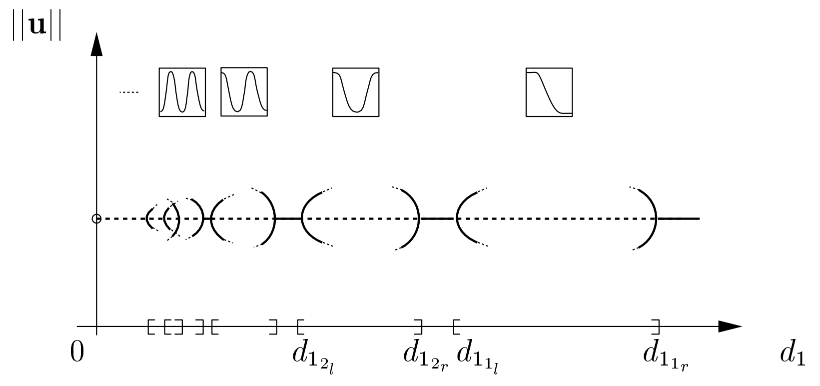

Figure 4: The branches of periodic solutions are sketched as maximal amplitude of the solution in an appropriate norm versus the diffusion coeffcient d_1 as bifurcation parameter. The mode destabilization is well separated up to the mode with the maximal number that can be estimated with Lemma 2.

3 A Model for Microbial Growth

The set-up of the kinetic system for growing cell cultures is derived from a chemostat model. Assume the microbes (algae or bacteria) to grow in a thin layer on top of an aggar (gel with nutrient) in a vessel, such that the nutrient is constantly supplied from below. Let $S := S(x,t)$ be the concentration of the growth-limiting nutrient within the layer. The dynamic is determined by the concentration of the fast growing (and therefore fast consuming) cell population $u := u(x,t)$, and $v := v(x,t)$ the slow growing cells. The concentrations are no longer constant but functions of space and time, since the model additionally considers a diffusion term for the cell movement. S^0 is the concentration of the limiting nutrient. For simplicity the dilution of the nutrient and the death rate of

the cells have the same constant rate D. But this assumption is only required to have a conservation of mass equation for the analysis. Computer algebra implementation of the equations show, that this can be considerably weakened: Dilution and death rate can have different values without changing the qualitative behavior of the solution set.
In the standard Monod model the specific growth rate f depends on the amount of supplied nutrient. It is modeled by a Michaelis-Menten term $f(S) = mS/(a+S)$, where m is the maximum growth rate and a is the half-saturation constant.
Dividing the cell culture into two phases that have different metabolisms gives rise to two different growth rates $f_1(S)$ and $f_2(S)$. Again they may be considered to be of the form $m_1S/(a_1+S)$ and $m_2S/(a_2+S)$, respectively. These terms are used for numerical investigations, although for the analysis of the model, f_1 as well as f_2 can be more general functions (see [7] and the references therein).
Assume $f_i,\ i = 1,2$ to be monotone increasing functions that saturate

(1) f_i are C^3 functions on an interval containing $[0,\infty)$.
(2) $f_i(0) = 0,\ f_i(S) \to m_i < \infty$ as $S \to \infty,\ m_1 > D > m_2$.
(3) $f_i'(S) > 0$ on $[0,\infty)$.

The change in metabolism is modeled by nonnegative functions $\alpha(S)$ and $\beta(S)$, and it is sufficient to have them continuous and piecewise differentiable. It can be assumed that $\alpha(S) = 0$ up to a threshold s_1 and that α is nondecreasing ($\alpha'(S) \geq 0$) for $S > s_1$. In contrast, β is nonincreasing ($\beta'(S) \leq 0$) for $0 \leq S < s_2$ and can be considered $\beta(S) = 0$ for $S \geq s_2$. Let α and β be piecewise linear functions of the form:

$$\alpha(S) = \begin{cases} 0 & & 0 \leq S < s_1 \\ K_1(S - s_1) & for & s_1 \leq S < s_1 + \varepsilon_1 \\ K_1\varepsilon_1 & & s_1 + \varepsilon_1 \leq S \end{cases}$$

$$\beta(S) = \begin{cases} K_2\varepsilon_2 & & 0 \leq S < s_2 - \varepsilon_2 \\ K_2(S - s_2) & for & s_2 - \varepsilon_2 \leq S < s_2 \\ 0 & & s_2 \leq S \end{cases}$$

with $K_1 > 0$ and $K_2 > 0$ positive constants.

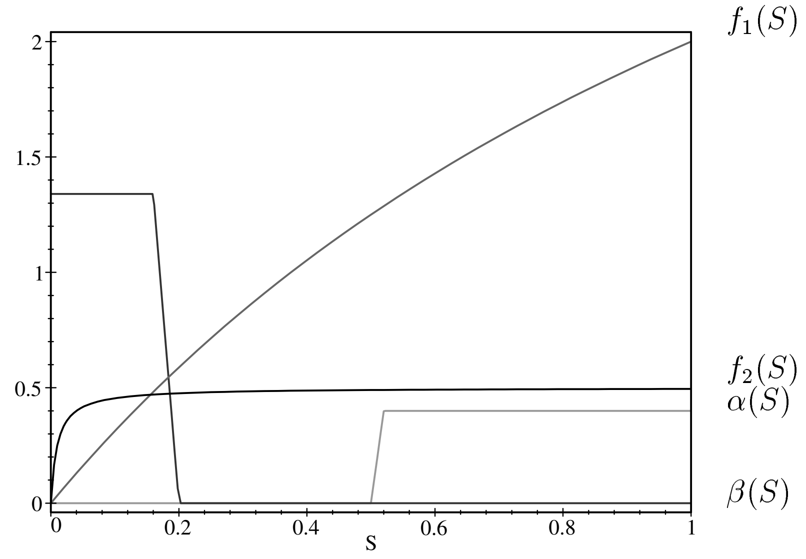

Figure 5: The functions $\alpha(S)$, $\beta(S)$, $f_1(S)$, and $f_2(S)$ are positive functions depending on the amount of nutrient S. The piecewise linear functions α and β model the exchange between both cell populations and are well separated by a gap in S, where both functions are identically zero.

Assume in the first place, that any of the variables may diffuse, but with different non-negative diffusion coefficients $d_i,\ i = 1, 2, 3$. The topmost layer of the aggar in the vessel is considered to be a one or at most two dimensional domain Ω with homogeneous Neumann, that is 'no flux', boundary conditions. The outer normal at the (smooth and convex) boundary of the domain is denoted by n.
This leads to the following model:

$$
\begin{array}{lcccccr}
\text{[Nutrient]} & \dfrac{\partial S}{\partial t} & = & d_1 \Delta S & + & (S^0 - S)D - f_1(S)u - f_2(S)v & \\
\text{[Phase 1]} & \dfrac{\partial u}{\partial t} & = & d_2 \Delta u & + & [f_1(S) - D - \beta(S)]u + \alpha(S)v & \text{in } \Omega \times (0,\infty) \\
\text{[Phase 2]} & \dfrac{\partial v}{\partial t} & = & d_3 \Delta v & + & [f_2(S) - D - \alpha(S)]v + \beta(S)u & \\
\end{array}
\tag{8}
$$

$$
\frac{\partial}{\partial n} S = \frac{\partial}{\partial n} u = \frac{\partial}{\partial n} v = 0 \qquad \text{on } \partial\Omega \times (0,\infty)
$$

with initial data (S_0, u_0, v_0) in $C(\Omega)$.
Existence of the solution for all time $t > 0$ results from bounded invariant rectangles, with the vectorfield of the kinetic equations at the boundary pointing strictly inwards (see for example Smoller [12], Chapter 14).

This model is biologically reasonable for the set of parameters consisting of positive constants. The analysis should be carried out for the whole range of positive parameters. The mathematical method of the previous section can now be applied in SECTION 4 and SECTION 5.

4 Transient Oscillations for Nongrowing Second Phase

Requirements for the wave bifurcation are at least one stablizing and one destablizing two dimensional submatrix of the kinetic system. The spatial operator appears exactly in the equation that does not belong to the stabilizing submatrix.
Here this result is applied to a simpler model than SYSTEM (8) in so far that it lacks a growth rate function for the cells of the second phase.

$$
\begin{aligned}
\frac{\partial S}{\partial t} &= d_1 \Delta S + (S^0 - S)D - f(S)u \\
\frac{\partial u}{\partial t} &= d_2 \Delta u + [f(S) - D - \beta(S)]u + \alpha(S)v \\
\frac{\partial v}{\partial t} &= d_3 \Delta v + [\qquad - D - \alpha(S)]v + \beta(S)u
\end{aligned}
\tag{9}
$$

Investigating the kinetic system means neglecting any explicit space dependence, looking at a single point in the vessel without exchange to the neighborhood, or at a mean value in a stirred vessel (see [7] for a detailed analysis which is sketches in the following). It is easy to see that the phase space $\mathbb{R}^3_+$ is positively invariant under the solution map of the kinetic system. Moreover it can be shown that the simplex $\{(S,u,v) \in \mathbb{R}^3_+ | S + u + v \leq S^0\}$ is positively invariant. Introducing a variable $z(t) = S(t) + u(t) + v(t)$ yields the differential equation $z' = (S^0 - z)D$; hence $z(t) \to S^0$ as $t \to \infty$.
The set of equilibrium points and their local (and global) stability behavior is known. $E^0 = (S^0, 0, 0)$ is an equilibrium point for all parameter values, and is globally stable in case of being the only fixpoint of the system. E^0 becomes a saddle point as soon as a second equilibrium point comes into play. This second point is either $E^u = (S^u, S^0 - S^u, 0)$ or $E^* = (S^*, u^*, v^*)$ that now is locally stable. A Poincaré-Bendixson argument together with Markus' theorem (see [9], and for the application of the argument see 5.2) state that there is no stable limit cycle and all trajectories are globally attracted by this second equilibrium except those on the boundary of the phase space.
The coexistence state of both cell populations in a stirred chemostat E^* is the only relevant equilibrium from which spatially nonconstant periodic solutions (standing wave) may bifurcate.
The Jacobian A at the stable equilibrium $E^* = (S^*, u^*, v^*)$ is given by

$$A|_{E^*} = \begin{pmatrix} -D - f'u^* & -f(S^*) & 0 \\ (f' - \beta')u^* & f(S^*) - D - \beta(S^*) & \alpha(S^*) \\ \beta' u^* - \alpha' v^* & \beta(S^*) & -D - \alpha(S^*) \end{pmatrix}$$

where $(\cdot)'$ denotes $\partial_S(\cdot)$ at E^*. Since E^* is an equilibrium point, and together with the conservation law $S^* + u^* + v^* = S^0$, this yields the following relations:

$$(u^* + v^*)D = (S^0 - S^*)D = f(S^*)u^* \quad \Rightarrow \quad \begin{aligned} v^*D &= (f(S^*) - D)u^* \\ [f(S^*) - D - \beta(S^*)]u^* &= -\alpha(S^*)v^* \\ [-D - \alpha(S^*)]v^* &= -\beta(S^*)u^* \end{aligned}$$

As a consequence, and since $v^* \neq 0$, $|A_{23}| = 0$ for all parameter values.

$$\begin{aligned} |A_{23}| &= [f(S^*) - D - \beta(S^*)][-D - \alpha(S^*)] - \alpha(S^*)\beta(S^*) \\ &= (f(S^*) - D)[-D - \alpha(S^*)] + \beta(S^*)D \\ &= \frac{v^*D(-\beta(S^*))}{v^*} + \beta(S^*)D = 0 \end{aligned}$$

Next it can be deduced that $|A_{12}| > 0$ for all parameter values.

$$|A_{12}| = \underbrace{(-D - f'(S^*)u^*)}_{<0}\underbrace{(f(S^*) - D - \beta(S^*))}_{\frac{-\alpha(S^*)v^*}{u^*} < 0} + f(S^*)\underbrace{(f'(S^*) - \beta'(S^*))}_{>0}u^* > 0$$

Assuming f to be a nondecreasing function and α to be positive, $|A_{13}|$ is always positive. There is no wave bifurcation possible for any positive values of diffusion coefficients. And also there is no Turing instability since there is no activator or destabilizing submatrix, even not in a single equation.

As a conclusion, this model will not exhibit any nontransient spatial structure neither stationary nor periodic, which bifurcates from a constant solution. Numerical tests for various initial conditions and parameters showed that there is no evidance for any spontaneous bifurcation away from constant solutions.

5 Sustained Oscillations

The question now is, what is required in the model to be able to describe also sustained spatial structure of the solution? Rovinsky, Menzinger [11] analyzed a three variable system with no activator submatrix but introduced a flow induced instability for spatial effects. Since there is no transport by flow in the vessel, this idea cannot be used as an explanation.

So the destabilization should be inhere in the dynamics. That means that there should be a destabilizing submatrix (see REMARK 5). Any entry on the trace of the Jacobian is nonpositive and there is no biological reason why this should be altered. That means that there is no activator equation. Also two dimensional submatrices have negative trace. Destabilizing two dimensional submatrices therefore have to have negative determinants. The model should be changed in a way to exhibit negative subdeterminants, but still being biologically reasonable, that is that growth rate functions are positive as well as increasing, and positive constants remain positive.
The slope of β in E^* can possibly make $|A_{12}|$ negative. Consider a z-shaped relation instead of a piecewise linear function such that E^* does not change its position but $\beta'(S^*) > 0$. Since α and β cannot be both nonzero, it follows that $\alpha(S^*) = 0$ in this case. Then this change of the slope of β additionally affects the stability of E^*, because $-\det A = -(-D)|A_{12}| < 0$. A negative subdeterminant yields a positive determinant and an essential condition, namely the stability of the equilibrium, is violated.

5.1 Slow Growing Second Phase

In SECTION 4 there was no growth rate function for the cells of the second phase, making some of the computations a bit easier. In fact introducing a positive growth rate also for this phase yields the possibility of negative $|A_{13}|$ without changing any of the qualitative results yet known about the chemostat model. SYSTEM (10) is the kinetic part of SYSTEM (8) and can be analyzed with the same methods as was sketched in the previous section. This kinetic model came up in discussions with Betty Tang in 1992 during her visit in Heidelberg.

$$\begin{aligned}\frac{\partial S}{\partial t} &= (S^0 - S)D - f_1(S)u - f_2(S)v \\ \frac{\partial u}{\partial t} &= [f_1(S) - D - \beta(S)]u + \alpha(S)v \\ \frac{\partial v}{\partial t} &= [f_2(S) - D - \alpha(S)]v + \beta(S)u\end{aligned} \tag{10}$$

Note that again introducing a variable $z(t) = S(t) + u(t) + v(t)$ yields the differential equation $z' = (S^0 - z)D$; hence $z(t) \to S^0$ as $t \to \infty$. There are at maximum two equilibrium points which are called again E^0 and the second E^u, if it is a pure fast growing cell culture, or E^*, the coexistence state.
They are found when solving the right hand side of SYSTEM (10) to be zero. This is equivalent to solving the following equations using the conservation law. Looking mainly for the coexistence state, it is the solution of

$$\begin{aligned}(S^0 - S)D - f_1(S)(S^0 - S - v) - f_2(S)v &= 0 \\ (f_2(S) - D - \alpha(S))v + \beta(S)(S^0 - S - v) &= 0\end{aligned}$$

with $v^* > 0$. Eliminating v from the equations yields

$$(S^0 - S)\left((D - f_1(S)) + \frac{(f_1(S) - f_2(S))\beta(S)}{D + \beta(S) - f_2(S)}\right) = 0$$

that then can be solved for S^*. Since $S^0 > S^*$ this is equivalent to solving $H(S^*) = 0$ with

$$H(S) := \begin{cases} \dfrac{(D - f_2(S))(D + \beta(S) - f_1(S))}{D + \beta(S) - f_2(S)} & \text{if} \quad 0 < S < s_2 \\ D - f_1(S) & \text{if} \quad S \geq s_2 \quad (\Leftrightarrow \beta(S) \equiv 0). \end{cases}$$

To avoid any unnecessary difficulties assume in the following always $f_2(S) < D$. This also excludes the case that $S^* \geq s_2$ as a by-product, because for nonzero v^* and $\beta(S^*) = 0$, the term $f_2(S) - D - \alpha(S)$ has to be zero to solve the equation, and that is not possible. The uniqueness of S^* (if it exists) is a consequence of the monotonicity of $D+\beta(S)-f_1(S)$. The set of equilibrium points consists again of either solely $E^0 = (S^0, 0, 0)$, or $\{E^0, E^u\}$, or $\{E^0, E^*\}$, respectively. E^0 is globally stable as long as it is the only attractor of the system. It becomes a saddle point when a second equilibrium occurs. In general the Jacobian is

$$A = \begin{pmatrix} -D - f_1' u^* - f_2' v^* & -f_1(S^*) & -f_2(S^*) \\ (f_1' - \beta')u^* + \alpha' v^* & f_1(S^*) - D - \beta(S^*) & \alpha(S^*) \\ (f_2' - \alpha')v^* + \beta' u^* & \beta(S^*) & f_2(S^*) - D - \alpha(S^*) \end{pmatrix}$$

and evaluated at E^0 it yields

$$A|_{E^0} = \begin{pmatrix} -D & -f_1(S^0) & -f_2(S^0) \\ 0 & f_1(S^0) - D - \beta(S^0) & \alpha(S^0) \\ 0 & \beta(S^0) & f_2(S^0) - D - \alpha(S^0) \end{pmatrix}.$$

The functions α and β cannot be both nonzero, so in either case ($\alpha = 0$ or $\beta = 0$) the eigenvalues of E^0 are just the entries on the diagonal

$$\Big\{-D,\ f_1(S^0) - D - \beta(S^0),\ f_2(S^0) - D - \alpha(S^0)\Big\}.$$

If there exists either E^u or E^* then $f_1(S^0) - D - \beta(S^0)$ is positive. This is because in either case $D + \beta(S) - f_1(S) = 0$ can be solved for $S^* < S^0$. For E^u it follows that $\beta(S^u) = 0$, for E^* it follows that $\beta(S^*) \neq 0$. Since β is nonincreasing and f_1 is strictly increasing it yields

$$D + \beta(S^0) \leq D + \beta(S^*) = f_1(S^*) < f_1(S^0)$$

and E^0 has one positive eigenvalue.

Now concentrate on E^* and its asymptotic stability. Therefore the symmetric functions are computed from the Jacobian at E^* where $\alpha(S^*) = 0$.

$$A|_{E^*} = \begin{pmatrix} -D - f_1'u^* - f_2'v^* & -f_1(S^*) & -f_2(S^*) \\ (f_1' - \beta')u^* & 0 & 0 \\ f_2'v^* + \beta'u^* & \beta(S^*) & f_2(S^*) - D \end{pmatrix}$$

It is easy to see that $\mathrm{tr}A < 0$, $\sum |A_{ij}| > 0$, and $\det A < 0$. In detail

$$\begin{aligned} |A_{23}| &= 0 \\ |A_{12}| &= f_1(S^*)(f_1' - \beta')u^* > 0 \\ |A_{13}| &= (-D - f_1'u^* - f_2'v^*)(f_2(S^*) - D) + f_2(S^*)(f_2'v^* + \underbrace{\beta'u^*}_{<0)}) \end{aligned}$$

and in the sum $\sum |A_{ij}|$ the only negative term $f_2(S^*)\beta'u^*$ is compensated by $f_1(S^*)(-\beta'u^*)$ of $|A_{12}|$. Finally every term in the determinant is negative

$$\det A = -f_2(S^*)(f_1' - \beta')u^*\beta(S^*) - \Big(-f_1(S^*)(f_1' - \beta')u^*(f_2(S^*) - D)\Big) < 0.$$

Therefore E^* is always a stable equilibrium point, if $-\mathrm{tr}A \sum |A_{ij}| > -\det A$, that is (C3). Since the computation now involves too many terms, there is an easier way to derive the asymptotic stability of E^* as a by-product of the next paragraph.

5.2 Asymptotic Behavior

The qualitative behavior of System (10) can be deduced from a planar autonomous system, so that any kind of chaotic behavior can be excluded. And also limit cycles can be excluded by a Poincaré-Bendixson argument. The idea is as follows: System (10) is equivalent to a nonautonomous system

$$\begin{aligned} \frac{\partial S}{\partial t} &= (S^0 - S)D - f_1(S)u - f_2(S)(z(t) - S - u) \\ \frac{\partial u}{\partial t} &= [f_1(S) - D - \beta(S)]u + \alpha(S)(z(t) - S - u) \end{aligned} \tag{11}$$

where $z(t)$ is the solution of $z' = (S^0 - z)D$. Since $z(t) \to S^0$ as $t \to \infty$, System (11) is asymptotically autonomous, with System (12) being the limit autonomous system.

$$\begin{aligned} \frac{\partial S}{\partial t} &= F_1(S, u) &= (S^0 - S)D - f_1(S)u - f_2(S)(S^0 - S - u) \\ \frac{\partial u}{\partial t} &= F_2(S, u) &= [f_1(S) - D - \beta(S)]u + \alpha(S)(S^0 - S - u) \end{aligned} \tag{12}$$

SYSTEM (12) is analyzed first. The triangular region $\Delta := \left\{(S,u) \in \mathbb{R}^2_+ | S + u \leq S^0\right\}$ is positively invariant. Equilibrium points are solely $\bar{E}^0 = (S^0, 0)$ or $\left\{\bar{E}^0, \bar{E}^u\right\}$ or $\left\{\bar{E}^0, \bar{E}^*\right\}$ with $\bar{E}^u = (S^u, S^0 - S^u)$ on the boundary of Δ and $\bar{E}^*$ inside.
Next it can be proven that $\bar{E}^0$ is a saddle whenever a second equilibrium exists. Therefore compute the Jacobian

$$J = \begin{pmatrix} -D - f_1'u^* - f_2'(S^0 - S^* - u^*) + f_2(S^*) & -f_1(S^*) + f_2(S^*) \\ (f_1' - \alpha' - \beta')u^* + \alpha'(S^0 - S^* - u^*) - \alpha(S^*) & f_1(S^*) - \alpha(S^*) - \beta(S^*) - D \end{pmatrix}$$

and evaluate it at $\bar{E}^0$:

$$J|_{\bar{E}^0} = \begin{pmatrix} -D + f_2(S^0) & -f_1(S^0) + f_2(S^0) \\ -\alpha(S^0) & f_1(S^0) - \alpha(S^0) - \beta(S^0) - D \end{pmatrix}$$

In case of $\alpha(S^0) = 0$ the eigenvalues are the entries on the diagonal. Since α is non-decreasing, $\alpha(S^*) = 0$ in this case, such that the same estimate holds as in the case of SYSTEM (10), and the second entry on the diagonal is positive.

$$D + \beta(S^0) \leq D + \beta(S^*) = f_1(S^*) < f_1(S^0).$$

In case of $\alpha(S^0) \neq 0$ it follows that $\beta(S^0) = 0$ and the eigenvalues compute to

$$\lambda_{1,2} = -D + \frac{1}{2}(f_1 - f_2 - \alpha) \pm \frac{1}{2}\sqrt{f_1^2 + 2(\alpha f_1 + f_1 f_2 - 3f_2\alpha) + \alpha^2 + f_2^2}.$$

If $f_2 = 0$ these are the eigenvalues already given in [7] namely $\{-D + f_1, -D - \alpha\}$ and it is obvious, that the first one is positive. However, it only grows as long as $2f_1 - 6\alpha + f_2 > 0$ because the expression under the square root is

$$(f_1 + \alpha)^2 + f_2(2f_1 - 6\alpha + f_2) > (f_1 + \alpha)^2$$

and the positive eigenvalue only increases when introducing a positive f_2.
The eigenvector of the stable manifold of $\bar{E}^0$ does not intersect the triangular region and the positive invariance guarantees that the ω-limit set of this region is either the other equilibrium or the union of the point and limit cycle(s).
By a Poincaré-Bendixson argument, the case of a limit cycles can be excluded. In short, any limit cycle of a planar system contains an equilibrium point of opposite asymptotic stability properties inside. Because of the positive invariance of the triangular region a limit cycle of SYSTEM (12) has to lie around the inner equilibrium E^*. Moreover, any limit cycle, if it exists, has to be stable. This can be shown when evaluating the divergence of the vectorfield along a periodic solution $\phi(t) = (S(t), u(t))$ of SYSTEM (12) with $\phi(0) = \phi(T)$, and $T > 0$ is the minimal period. The second equation yields $f_1 - D - \beta = \dfrac{\partial_t u - \alpha v}{u}$,

and integration by time is always negative.

$$\begin{aligned}\int_0^T \left(\frac{\partial F_1(\phi(t))}{\partial S} + \frac{\partial F_2(\phi(t))}{\partial u}\right) dt \quad &= \quad -\int_0^T \Big(D - f_2(S(t))\Big)\, dt \\ &\quad -\int_0^T \Big(f_1'(S(t))u(t) + f_2'(S(t))(S^0 - S(t) - u(t))\Big)\, dt \\ &\quad -\int_0^T \frac{\alpha(S(t))(S^0 - S(t))}{u(t)}\, dt \quad + \quad \log\frac{u(T)}{u(0)} \\ &< \quad 0\end{aligned}$$

If $\bar{E}^*$ is asymptotically stable, no limit cycle can occur, since at least one unstable cycle has to be inside Δ. To see the asymptotic stability of $\bar{E}^*$ first realize that $\alpha(S^*) = 0$. Assuming $\alpha(S^*) \neq 0$ it follows that $\beta(S^*) = 0$ and from $F_2(S, u) = 0$ it follows $(f_1 - D)u = -\alpha(S)(S^0 - S - u)$. This leads to a contradiction when solving $F_1(S, u) = 0$

$$\begin{aligned}&(S^0 - S - u)D + uD - f_1 u - f_2(S^0 - S - u) = 0 \\ \Leftrightarrow \qquad &-(f_1 - D)u = (f_2 - D)(S^0 - S - u)\end{aligned}$$

since $f_1 - D$ cannot be negative and positive. So $f_1 = D$ and $\alpha(S^*) = 0$.
Knowing that $\alpha(S^*) = 0$ (but in fact $\beta(S^*) \neq 0$ and $f_1(S^*) - \beta(S^*) - D = 0$), it is easy to verify the stability of $\bar{E}^*$, since $J|_{\bar{E}^*}$ has negative trace and positive determinant.
Now Markus' Theorem can be applied showing immediately that every trajectory of the nonautonomous system with initial value inside Δ asymptotically approaches $\bar{E}^*$. Finally all trajectories of System (10) approach an equilibrium point E^0, E^u or E^* since it is equivalent to the nonautonomous system as can be seen with the help of the conservation law.

5.3 Requirements for Wave Bifurcation

There is no difference in the global behavior of the solution map of a three variable chemostat model with or without a growth rate function $f_2(S)$ as can be seen from the study of the asymptotic behavior of System (10). But the Jacobian at E^* now has a destabilizing two dimensional submatrix. Recall that $|A_{13}|$ includes a negative term, namely $\beta' u^*$. Using the computer algebra program Maple gave a first hint, that there exists a stable equilibrium point E^* with a negative $|A_{13}|$. Since $|A_{12}|$ remains positive for all parameter values, applying Remark 5 identifies the third variable to be the diffusive species. Recall the formal limit (Equation (2)) but with the nonzero diffusion coefficient in the third variable.

$$\tilde{\Delta}_2(0, 0, d_3\mu_m) \quad = \quad -\mathrm{tr}(A_{12})(d_3\mu_m)^2 + (|A_{13}| + |A_{23}| + \mathrm{tr}(A_{12})\mathrm{tr}A)d_3\mu_m + \Delta_2$$

With the appropriate cyclic changes the requirements for the kinetic system are as follows:

(C4) $-\mathrm{tr}(A_{12}) > 0$
(C5) $-(|A_{13}| + |A_{23}| + \mathrm{tr}(A_{12}))\mathrm{tr}A) > 0$
(C6) $(|A_{13}| + |A_{23}| + \mathrm{tr}(A_{12})\mathrm{tr}A)^2 + 4\mathrm{tr}(A_{12})\Delta_2 > 0$

Then it is possible that the parabola is negative for some $d_3\mu_m$, that is $\tilde{\Delta}_2(d_3\mu_m) < 0$. Spatial inhomogeneities in the initial conditions now give rise to time-periodic nonconstant solutions such as standing waves. The solution branches for the various spatial wavelengthes bifurcate from the stable constant solution for critical diffusion parameters that can be determined by LEMMA 2. So the system may have two (or more) stable attractors, namely a constant steady state and a nonconstant time-periodic solution.

5.4 Steady State Turing Patterns

The second variable has to be the diffusive species for steady nonconstant solutions as can be seen from EQUATION (4) adepted to the case of diffusion in the second variable.

$$-\mathrm{det}\tilde{A}(0, d_2\mu_m, 0) = |A_{13}|d_2\mu_m - \mathrm{det}A$$

Again this limit can be negative for some $d_2\mu_m$. In this case the spatial inhomogeneities of initial conditions give rise to patches where either phase 1 or phase 2 of the cell culture predominates.
Recall the case of a two variable activator-inhibitor system, where (c3) guarantees the existence of an activator, but the inhibitor has to diffuse faster. It can be observed, that for three variables and in the formal limit with only one nonzero diffusion coefficient, the now two dimensional submatrix is a destabilizing activator system and the remaining inhibitor equation includes the spatial operator.

6 Numerical Investigations

A sustained oscillation in space and time for SYSTEM (8) could as well be verified numerically.
First of all the stable equilibrium point E^* had to be detected with the help of a computer algebra program (Maple). For the following set of parameters

$$S^0 = 1.0,\ D = 0.5,\ m_1 = 5.0,\ m_2 = 0.5,\ a_1 = 1.5,\ a_2 = 0.01,$$
$$K_1 = 20.0,\ K_2 = 33.5,\ s_1 = 0.5,\ s_2 = 0.2,\ \varepsilon_1 = 0.02,\ \varepsilon_2 = 0.04$$

the equilibrium point $(S^*, u^*, v^*) \approx (0.19755, 0.18241, 0.62003)$ satisfies (C1) – (C3) and is therefore an asymptotically stable constant solution. The conditins (C4) – (C6) are as well fulfilled for these parameters. With LEMMA 2 the maximal wavenumber can be estimated to be either seven or eight. Critical diffusion coefficients for the seventh mode are round about $d_{3_{7_l}} = 0.001514$ and $d_{3_{7_r}} = 0.0018998$ and for the eighth mode $d_{3_{8_l}} = 0.001159$ and $d_{3_{8_r}} = 0.001455$. The destabilization of the nineth and tenth mode overlap, since

the diffusion coefficient lies in $d_{3_9} \in (0.000917, 0.001149)$ and $d_{3_{10}} \in (0.000742, 0.000931)$, respectively.
The discretized system of ordinary differential equations was solved with the LSODE package (see [6]), using numerical estimates of Jacobian matrix. The error tolerances were 1×10^{-8} relative and 1×10^{-12} absolute. Time discretization was done implicitly with a maximal step size of $t = 0.01$. With the above considerations a diffusion coefficient $d_3 = 0.001$ destabilizes the nineth mode and not yet the tenth. To guarantee the spatial step size to be of the appropriate order of magnitude, i.e. $h^2 \sim t$, the space discretization is 300 gridpoints on a unit interval, so $h^2 = d_3\,^{-1}(300)^{-2} \sim 0.01$.
The initial data was taken close to E^* with inhomogeneous perturbation within the spatial wavelength and of an amplitude of approximately 0.01 as a single peak in each equation at the same place but arbitrarily located on the grid. Homogeneous Neumann or 'no flux' conditions served as boundary conditions.
It could be observed that the small initial peak grows by the dynamic to a peak of considerable height before the diffusion broadened it. This maximal amplitude was used for scaling purposes over the whole time scale. The wave pattern traveled to the boundary, was reflected and decayed in amplitude. But finally when the predicted stable pattern was approximately reached, the wave grew again in amplitude. The computed result was the predicted standing wave pattern of wavenumber 9 (see FIGURE 6).
Qualitatively the same attractor results from randomly disturbed initial data having mean value at the constant solution E^*.

7 Discussion

The modified Monod model of [7] turned out to be very robust against changes that allow for spatially nonuniform perturbations. The local and global solution set cannot be altered in their asymptotic behavior although the transient growth dynamics gain complexity.
The standing wave solution won't be there until the quiescent cells have a growth rate modeled by a positive increasing function $f_2(S)$. Now the two dimensional submatrix of the Jacobian consisting of the derivatives of nutrient and of these slow growing cells is destabilizing. Adding a diffusive term to this third equation of cells of phase 2 folds up a periodic nonconstant solution. For the vessel, no flux boundary conditions might be the most appropriate. So the initial wave gets reflected at the boundary and a standing wave solution is established.
Numerically the only asymptotically (or oribitally) stable solutions to be found are the constant E^* and this standing wave solution. If the initial data stimulates a wavelength greater than the predicted one, the smoothing effect of the diffusion will win against the dynamic and the constant solution survives.
Note here, that without diffusion, no such periodic solution exists. That means stirring the reactor would always lead to a constant steady solution of either no surviving cells, a pure fast growing cell culture, or a coexistence state.

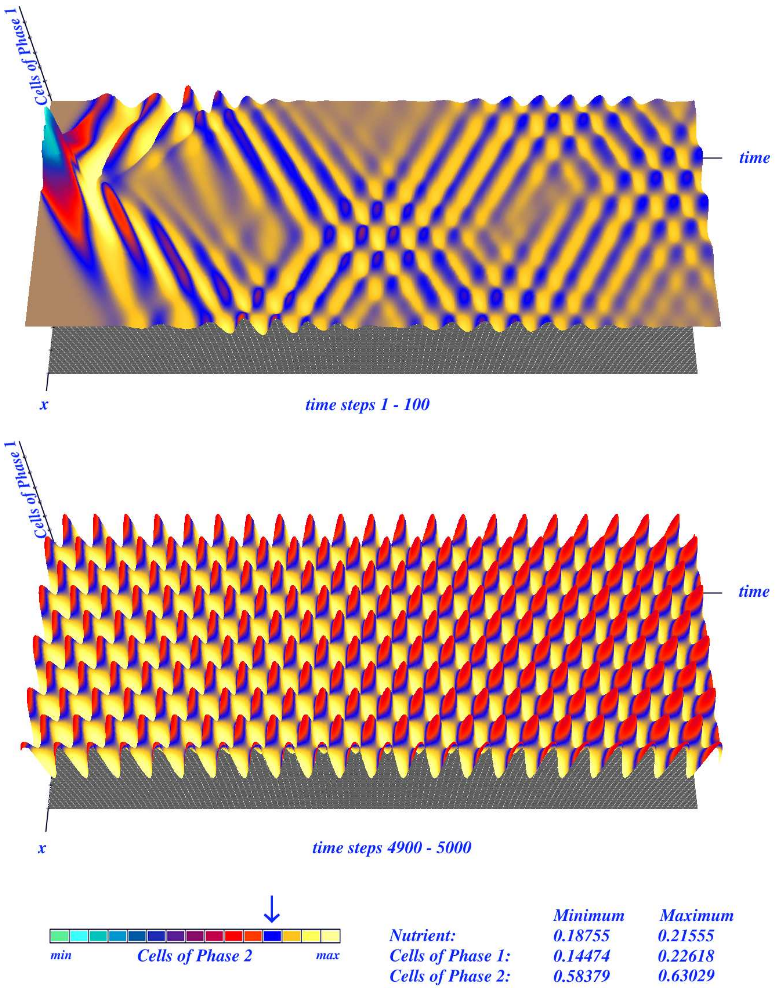

Figure 6: The temporal evolution of a 1-D spatially nonuniform initial data (above, time steps 1-100) to a standing wave pattern (below, time steps 4900-5000) show the concentration of slow growing but mobile cells mapped in color to the profile of fast growing and immobile cells. The value for the constant solution of slow growing cells v^* is marked with a dark color (see the arrow).

If the experiment shows sustained spatio-temporal oscillations, the mathematical model should contain a growth rate function smaller than the death rate D for the cells of the second phase and these cells should have the property to diffuse through the medium (gel) in the vessel. The result can be interpreted in the way that the cells do not only change their metabolism but also change their mobility for example by shrinking to a size that allows for diffusion, whereas the fast growing cells stick to their places and are immobile.

If the experiment shows a patchiness, the same mathematical model can be used but here the fast growing cells should have the greater mobility.

To model transient oscillations, there was the need of separating the cells in active and quiescent ones. For sustained oscillations it is now required that the cells of phase 2, which have the smaller growth rate, can diffuse. This is sufficient for a stable standing wave, that would disappear again in case that the vessel is stirred.

This paper is dedicated to the memento of Betty Tang.

References

[1] H. Amann, Hopf Bifurcation in quasilinear reaction-diffusion systems, Proc. Claremont Conf. on Diff. Eq. and Appl. to Biol. and Pop. Dyn., S. Busenberg (ed.), (1990).

[2] A. Cunningham, P. Maas, Time lag and nutrient storage effects in the transient growth response of Chlamydomonas reinhardii in nitrogen-limited batch and continuous culture, J. Gen. Microbiol. 104: 227 - 231 (1978).

[3] M. Crandall & P. Rabinowitz, The Hopf Bifurcation Theorem in Infinite Dimensions, Arch. Rational Mech. Anal. **67**, 53 - 72, (1977).

[4] H. Fujii, M. Mimura, Y. Nishiura, A Picture of the Global Bifurcation Diagram in Ecological Interacting and Diffusing Systems, Physica 5D, 1 - 42, (1982).

[5] F. R. Gantmacher, Applications of the Theory of Matrices, Interscience Publishers, Inc., New York, (1959).

[6] A. C. Hindmarsh, ODEPACK, A Systemized Collection of ODE Solvers, Scientific Computing, North-Holland, Amsterdam, 55 - 64, (1983).

[7] W. Jäger, S. Krömker, B. Tang, Quiescence and Transient Growth Dynamics in Chemostat Models, Math. Biosciences **119**, 225 - 239, (1994).

[8] S. Krömker, Wave Bifurcation, Proceedings of the 3rd Workshop on Modelling of Chemical Reaction Systems, Editors: F. Behrendt, W. Jäger, J. Warnatz, (1996).

[9] L. Markus, Asyptotically autonomous differential systems, Contributions to the theory of Nonlinear oscillations, Vol. III, S. Lefschetz, ed. Princeton Univ. Press, Princeton, 17 - 29, (1956).

[10] J. D. Murray, Mathematical Biology, Biomathematics Vol. 19, Springer-Verlag, 2nd print. (1990).

[11] A. B. Rovinsky, M. Menzinger, Differential Flow Instability in Dynamical Systems without an Unstable (Activator) Subsystem, Phys. Rev. Letters, Vol. 72, No. 13, p. 2017, (1994).

[12] J. Smoller, Shock Waves and Reaction-Diffusion Equations, A Series of Comprehensive Studies in Mathematics 258, Springer-Verlag (1983).

[13] A. M. Turing, The Chemical Basis of Morphogenesis, Philos. Trans. R. Soc. London, Ser. B **237**, 37 (1952).