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A delay model for quorum sensing of *Pseudomonas putida*☆

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ABSTRACT

The bacterial strain *Pseudomonas putida* IsoF, isolated from a tomato rhizosphere, possesses a quorum sensing regulation system, which allows the bacteria to recognise aspects of their environment or to communicate with each other by the so-called autoinducer molecules. In an experimental study, the time series of the autoinducer production did not show the expected behaviour, as it was observed for other bacterial species by indirect measurements.

The modelling approach introduced here allows an explanation of the behaviour, supporting the hypothesis of the existence of a further (not yet detected) enzyme, which degrades the autoinducer into an inactive form. Especially the properties of the considered delay differential system allow for the description of the time series. For example the appearance of a first small maximum in the initial phase can be explained by a delay differential equation.

1. Introduction

Originally, the concept of quorum sensing (QS) was introduced as a possibility for the bacteria to control the expression of genes in response to their own cell density (Fuqua and Greenberg, 2002). Bacterial cells produce and release autoinducer molecules into their own environment, i.e. the extracellular autoinducer concentration depends on the present cell density. When a certain threshold concentration is exceeded, the bacteria change their behaviour by gene transcription induction. Additionally, the environment itself (e.g. the diffusible space around a single cell Redfield, 2002 and spatial effects like clustering of cells) influences the autoinducer concentration and by that the behaviour of the bacteria. This observation leads to the more general concept of "Efficiency sensing" (Hense et al., 2007). Many bacterial species use such regulation systems (see e.g. Fuqua and Greenberg, 2002; Gray and Garey, 2001; Miller and Bassler, 2001; Waters and Bassler, 2005; Whitehead et al., 2001).

An important bacterium on the ecological level is *Pseudomonas putida*. As many root-colonising pseudomonads, it belongs to the so-called plant-growth promoting bacteria (Steidle et al., 2002). Besides the plant growth promotion activity, *P. putida* inhibits plant pathogens and contributes to the degradation of toxic organic compounds.

In *P. putida* strain IsoF and other Gram-negative bacteria, the autoinducer molecules belong to the family of acyl homoserine lactones (AHL). These are produced by the so-called AHL synthases, e.g. members of the so-called LuxI family (Greenberg, 1997; Hastings and Greenberg, 1999). Also the PpuI in *P. putida* IsoF is of such a type, whereas the response to local concentrations of AHLs is mediated here (with a positive feedback loop) by the transcriptional activator PpuR, a LuxR-homologue protein (Steidle et al., 2001). In Steidle et al. (2002), it was indeed shown that, for *P. putida* IsoF at least four kinds of AHLs are synthesised by PpuI. However, the different AHLs have all the *ppu* system as AHL-dependent quorum sensing locus and the quorum sensing cascade does not change from one AHL type to the other.

Starting from the situation presented in Fekete et al. (2010), with this work we analyse the same system in greater detail, giving particular attention to the AHL dynamics in the initial phase of the experiment. The results in Fekete et al. (2010) gave a strong hint about the existence of an AHL-degrading enzyme. Even though it was not really shown to which family the putative AHL-degrading enzyme belongs, it is reasonable to assume it is a lactonase (for details see Fekete et al., 2010) and we will introduce this term to describe the additional AHL-degradation in the system.

Addition of AHL (spiking) in the *P. putida* IsoF growing medium (Fekete et al., 2010) showed an increase in the amount of the hydrolysis product after 1–2 h. For a better description and understanding of the QS system, it seems reasonable to include a delay also in the mathematical model, which is done in Section 3, resulting in a better fit to the experimental data.

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In the initial phase of the experiment, short after the bacteria were taken from the pre-culture, a maximum with lower amplitude in the AHL concentration was observed. This can be qualitatively explained by influences from the pre-culture on the bacteria. The cells might still include a high number of PpuR-AHL complexes, leading to a quite fast production of AHL. But in the new system, the complexes may dilute faster than being rebuilt, due to the relatively low cell density during that time, which results in a decrease of AHL production, probably also due to the degradation activity in the growing medium. Only when the bacterial population has a certain density, the PpuR-AHL threshold concentration can be reached again. Consequently, a second (larger) maximum in the AHL-concentration appears, followed by a fast decrease as a result of the lactonase activity.

2. Basics

2.1. Experimental Setting

The underlying experimental procedure was performed as described in Fekete et al. (2010). Briefly: Cultures of strain IsoF were grown in minimal medium buffered to a pH of 6.8, to avoid abiotic degradation of AHLs. Eighteen flasks containing 200 ml of medium each were prepared for the experiment.

Bacteria were inoculated to all the flasks at the same time and let grow. Samples were taken right after the inoculation and then every hour. Every time 10 μl of medium were fixed in a glass and prepared for cell counting under microscope (additionally, the OD was measured). The collected 200 ml samples were centrifuged for 15 min and the supernatant was used for a further purification of the AHLs from the other present components, applying solid phase extraction (SPE) which also allows to concentrate the analytes (clean-up).

Afterwards the solution was analysed by ultra performance liquid chromatography, in which the substances are separated within 1.5 min. The retention time and UV–vis spectrum of the peak were applied as identification criteria, and the peak area was used for quantification applying a calibration curve. The whole method was validated for correctness (Li et al., 2006).

2.2. Introduction of the Model

As a first approach, we want to use a system of ordinary differential equations (ODEs) and check if this is sufficient to explain the experimental findings.

The growth of the bacterial population is assumed to be logistic, which is confirmed by the experimental data. For the AHL regulation system, we neglect the dynamics of PpuI, assuming that PpuI and AHL are in equilibrium. In contrast to the approach e.g. in Kuttler and Hense (2008), we do not consider here an explicit equation for the polymerised complexes of PpuR-AHL, but assume that the complexes and the polymers thereof are in equilibrium. This allows us to reduce the number of equations. *P. putida* IsoF can produce different types of AHL, but as 3-oxodecanoyl-homoserine lacone (OC10) seems to be the main type, we restrict ourselves to that and neglect the others (Fekete et al., 2010).

We do not make any distinction between AHL molecules outside the cells and AHLs inside the cytoplasm, but simply consider the total AHL concentration in the system. Actually, diffusion through the cell membrane plays an important role in regulation processes; however AHLs can diffuse into and out of the cell without necessitating any transport mechanisms and the diffusion process goes pretty fast, compared to the time scale chosen for the experimental measurements (1 h). Moreover it is a fact that no AHL molecule was found inside the pellets; this let us suppose once more that the diffusion through the cell membrane occurs within a few minutes

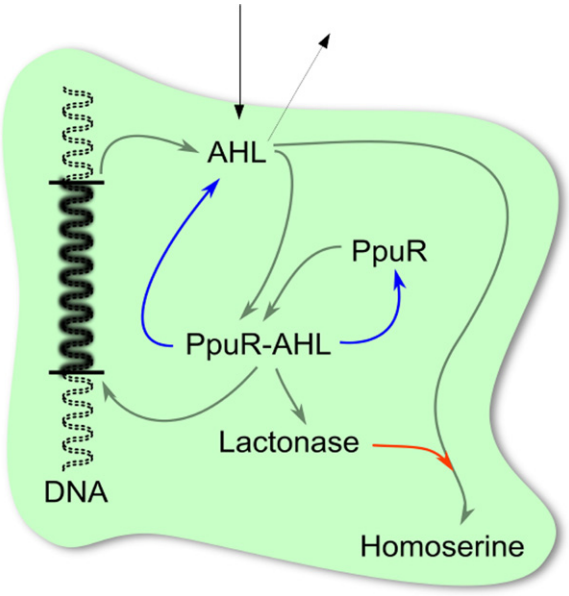

Fig. 1. Sketch of the mathematical model for the regulation processes in one *Pseudomonas putida* IsoF cell.

(or anyway less than the time it takes to centrifuge and clean-up the cells).

As usual in similar systems, the complex PpuR-AHL controls the production of AHL, including a positive feedback.

Also the production of the lactonase is assumed to be controlled by PpuR-AHL, similarly to the production of AHL via PpuI.

For PpuR itself, we hypothesise an independent production, ignoring a possible weak feedback as it was found e.g. for LuxR (Goryachev et al., 2006), because the influence thereof is not relevant for our purpose here.

A sketch of the mathematical model is shown in Fig. 1. Considering the notation for variables and parameters given in Tables 1 and 2, we define the following equations as basic system:

$$\dot{N}(t) = a\left(1 - \frac{N(t)}{K}\right)N(t), \tag{1}$$

$$\dot{A}(t) = \left(\alpha + \beta\frac{R_A(t)^{n_1}}{R_{A1}^{n_1} + R_A(t)^{n_1}}\right)N(t) - \gamma A(t) + \gamma_3 R_A(t) - \alpha_1\frac{R(t)A(t)}{N(t)} - K_E A(t)E(t), \tag{2}$$

$$\dot{R}_A(t) = \alpha_1\frac{R(t)A(t)}{N(t)} - \gamma_1 R_A(t) - \gamma_3 R_A(t), \tag{3}$$

$$\dot{R}(t) = \alpha_R N(t) + \gamma_3 R_A(t) - \alpha_1\frac{R(t)A(t)}{N(t)} - \gamma_R R(t), \tag{4}$$

$$\dot{E}(t) = \alpha_E\frac{R_A(t)^{n_2}}{R_{A2}^{n_2} + R_A(t)^{n_2}}N(t) - \gamma_E E(t). \tag{5}$$

The experimental data for AHL show two maxima: a smaller one, after few hours, and a larger one, after approximately 10 h from

Table 1
Notations for the variables of the basic ODE system (1)–(5).

Name	Description
N	Bacterial population density
A	Concentration of AHL (autoinducer)
R	Concentration of PpuR (receptor)
R_A	Concentration of the PpuR-AHL complex
E	Concentration of the lactonase (enzyme)

Table 2
Notations for the parameters of the basic ODE system (1)–(5).

Name	Description
a	Reproduction rate for bacteria
K	Capacity of the environment
α	AHL background production rate
β	AHL PpuR-AHL-dependent production rate
n_1	Polymerization degree for PpuR-AHL
R_{A1}	Threshold value for PpuR-AHL (AHL production)
γ	AHL abiotic degradation rate
γ_3	Complex dissociation rate
α_1	Binding rate for PpuR-AHL
K_E	AHL lactonase-dependent degradation rate
γ_1	PpuR-AHL degradation rate
α_R	PpuR background production rate
γ_R	PpuR abiotic degradation rate
α_E	Lactonase production/activation rate
n_2	Polymerization degree for PpuR-AHL (in the lactonase activation)
R_{A2}	Threshold value for PpuR-AHL (in the lactonase activation)
γ_E	Lactonase abiotic degradation rate

the beginning of the experiment. The fast decrease of AHL after the second maximum can be explained by the lactonase hypothesis (see Fekete et al., 2010), which is already included in our model.

Using suitable parameter values, as next we investigate if the model can also display the first maximum in a reasonable way. That may give a hint on the reliability of this unusual time series for the autoinducer production.

2.3. Estimation of Parameter Values

As the reader may easily understand, our mathematical model has several degrees of freedom, due to the large number of parameters and the not too many experimental data available. Experimental data were only obtainable for bacterial population and AHL concentration. As next we aim to give a first approach of these data by fitting the parameter values present in the model. The achievement of plausible parameter values helps further for the validation of the modeling approach. In the following, parameter values will be fitted according to the mean square error algorithm.

First, the growth of the bacterial population is considered. Fitting the logistic growth curve to the experimental data yields values for the parameters a and K, see Fig. 2(a).

The (abiotic) AHL degradation was examined in detail in Englmann et al. (2007), so we can just obtain a suitable parameter value from there. Concerning the AHL production, suitable parameter values for α, β, γ, n_1 and R_{A1} can be taken from the approach in Fekete et al. (2010). The determination of parameter values for PpuR and the complex PpuR-AHL is more difficult, since no direct experimental data are available for that. For an estimate, we assume that one bacterial cell of *P. putida* IsoF contains approximately 500 PpuR molecules (according to the finding that approximately 500 LuxR molecules are contained in one cell of *Vibrio fischeri*; Kolibachuk and Greenberg, 1993).

Table 3
Chosen values for the parameters of the ODE system (1)–(5).

Parameter	Value	Parameter	Value
a	0.57 1/h	γ_1	0.5 1/h
K	7.3907×10^{11} cells/l	γ_3	0.09 1/h
α	2.3×10^{-19} mol/(cells h)	α_R	4.15×10^{-21} mol/(cells h)
β	2.3×10^{-18} mol/(cells h)	γ_R	0.005 1/h
γ	0.005545 1/h	α_E	3×10^{-9} mol/(cells h)
n_1	4.5	R_{A2}	1.3×10^{-10} mol/l
R_{A1}	1.2×10^{-10} mol/l	γ_E	0.5 1/h
α_1	6×10^{16} cells/(mol h)	n_2	9
K_E	0.65 l/(mol h)		

The best fitted parameter values are summarised in Table 3 and the corresponding solution is shown in Fig. 2(b).

3. A Delay Model for Quorum Sensing of *Pseudomonas putida* IsoF

As the reader will have noticed from Fig. 2(b), the ordinary differential equation system is not enough to explain the existence of an initial peak in the AHL concentration. A reason for this, is possibly due to the fact that in the given ODE model, the enzyme could (depending on the PpuR-AHL concentration) be active from the very beginning and thus contribute to the degradation of AHL molecules. But as emerged from supplementary spiking tests (data not shown here) the AHL decay starts actually only after a couple of hour from the beginning of the experiments.

Therefore we decided to introduce a time delay for the activation of the lactonase. We define a delay $\tau > 0$ to express the effect of further regulatory processes for the lactonase, which were not explicitly considered in the model.

The standard form of a DDE problem is given by:

$$\dot{y}(t) = f(t, y(t-\tau_1), \ldots, y(t-\tau_n)), \quad t \geq t_0 \tag{6}$$

$$y(t) = \phi(t), \quad t \leq t_0. \tag{7}$$

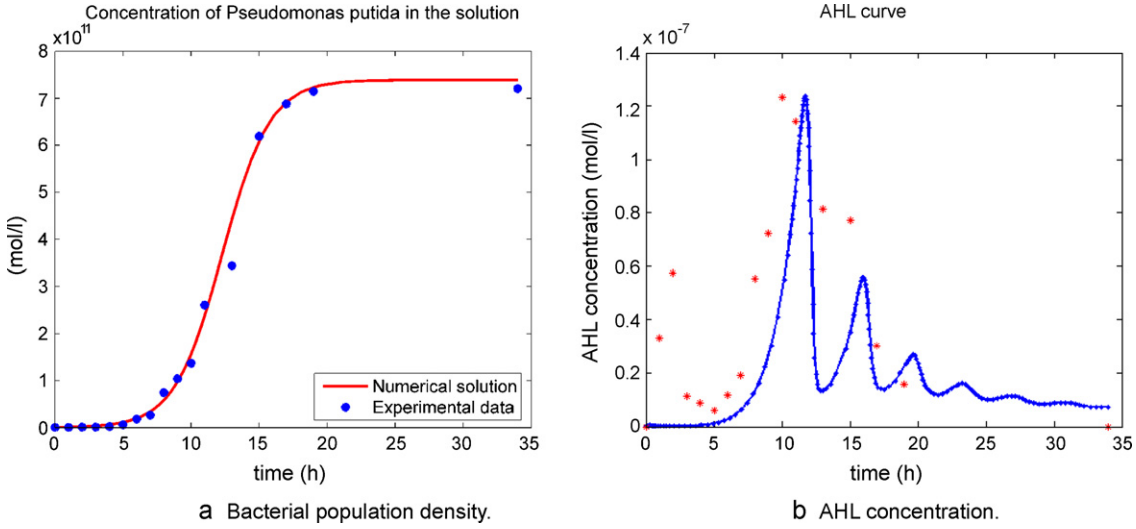

Fig. 2. Experimental data and numerical results: (a) bacterial population and (b) AHL concentration.

Table 4
Constant values used for the definition of (9).

History function ($t<0$) of the delay system
$\tilde{N} = 6.54 \times 10^{8}$
$\tilde{A} = 3.699 \times 10^{-10}$
$\tilde{R_A} = 1.071 \times 10^{-7}$
$\tilde{R} = 8.631 \times 10^{-9}$
$\tilde{E} = 1.233 \times 10^{-11}$

According to the complexity of the problem, the always non-negative delays τ_i may be constant values, or functions of t, or functions of t and y itself (Bellen and Zennaro, 2003).

The *history function* $\phi(t)$ is at least defined in $[r, t_0]$, where

$$r = \min_{1\le i\le n} \{\min_{t\ge t_0}(t - \tau_i)\}.$$

We couple the ODE system (1)–(4) with a constant delay equation for the lactonase concentration:

$$\dot{E}(t) = \alpha_E N(t) \frac{R_A(t-\tau)^{n_2}}{R_{A2}^{n_2} + R_A(t-\tau)^{n_2}} - \gamma_E E(t). \tag{8}$$

As a consequence of the delay τ, the variation of the enzyme concentration at time t depends on the PpuR-AHL concentration at time $t-\tau$. Biologically, this means that the lactonase is produced/activated by bacteria just after "a certain time" (whereas AHL, PpuR and PpuR-AHL are synthesized almost immediately), depending on the concentration of the complex. One possible explanation for the delay is that several steps are necessary for the production/activation of the lactonase by the cells.

Concerning the history function of the system, for simplicity we define:

$$\phi(t) \equiv (\tilde{N}, \tilde{A}, \tilde{R_A}, \tilde{R}, \tilde{E}) \quad \text{for} \quad t \le 0, \tag{9}$$

where the values $(\tilde{N}, \tilde{A}, \tilde{R_A}, \tilde{R}, \tilde{E})$ are constants larger or equal than zero (see Table 4). The reason for such a choice is the pre-culture in which bacteria were grown. Indeed, in the pre-culture bacterial cells contained PpuR and already produced AHL, leading to PpuR-AHL formation.

The parameter values given in Table 5, together with the history function (Table 4) have been used for the numerical simulation of the solutions of the system. The AHL curve obtained with these values is shown in Fig. 3.

4. Analyse the Models

4.1. Existence and Uniqueness of Solutions

For the correctness of the mathematical model, we have to prove existence and uniqueness of solutions for both the ordinary differential equations and the delay models.

Recalling the theory of ODE systems (see e.g. Perko, 2001), it is trivial to show existence and uniqueness of the solutions of (1)–(5)

Table 5
Parameter values used for the simulation of the delay system.

Parameter	Value	Parameter	Value
a	0.57 1/h	γ_1	0.5 1/h
K	7.3907×10^{11} cells/l	γ_3	0.04 1/h
α	2.338×10^{-18} mol/(cells h)	α_R	1×10^{-19} mol/(cells h)
β	5.128×10^{-17} mol/(cells h)	γ_R	0.005 1/h
γ	0.05 1/h	α_E	1.6515×10^{-9} mol/(cells h)
n_1	10	R_{A2}	1.048×10^{-7} mol/l
R_{A1}	1.048×10^{-7} mol/l	γ_E	0.05 1/h
α_1	6.5×10^{19} cells/(mol h)	n_2	5
K_E	0.35 l/(mol h)	τ	2 h

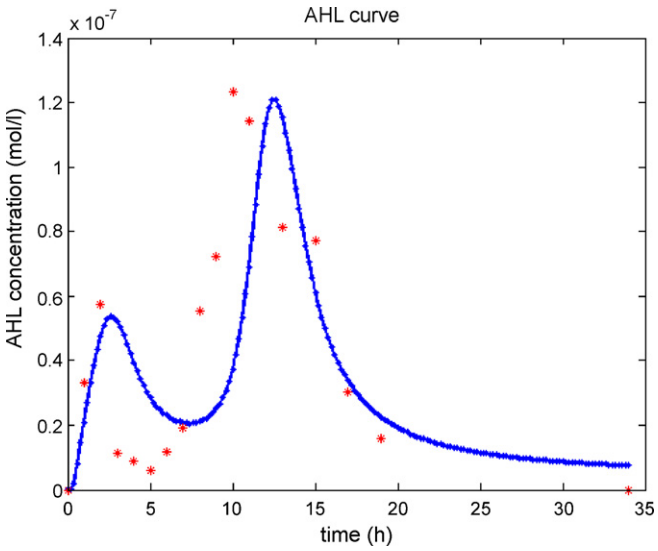

Fig. 3. Numerical solutions of the delay problem.

by verifying the C^1-continuity of the right hand-side of the problem (details can be found in Barbarossa, 2008). A bit more effort is necessary, when investigating the delay system (1)–(4), (8).

Consider the general time dependent delay problem:

$$\dot{y}(t) = f(t, y(t), y(g_1(t)), \cdots, y(g_m(t))),$$
$$t - r \le g_i(t) \le t \qquad \text{for} \quad t \ge t_0, \tag{10}$$

$$y(t) = \phi(t), \quad t_0 - r \le t \le t_0, \tag{11}$$

where $f : [t_0, t_f] \times D^m \to \mathbb{R}^d$ with D^m an open subset of $\mathbb{R}^d$ is given (Driver, 1977).

Analogously to the ODE case, existence and uniqueness of solutions for a delay problem are essentially based on the continuity and Lipschitz continuity of the right-hand side $f(t, u, v)$.

Denote with $\mathcal{C}$ the set $C([-r, 0], \mathbb{R}^n)$ of all continuous functions from $[-r, 0]$ in $\mathbb{R}^n$.

For simplicity, we introduce

$$F(t, y_t) := f(t, y(t), y(t - \tau(t))), \tag{12}$$

with $F : [t_0, t_f] \times \mathcal{C} \to \mathbb{R}^n$, which allows us to consider the *functional delay differential equation*, instead of a delay problem.

In literature, it is usually assumed that the functional F is continuous on $[t_0, t_f] \times \mathcal{C}$, meaning that way the continuity with respect to t and ϕ_t. The reader may anyway notice, that we deal with an autonomous problem, and therefore no continuity with respect to t is needed.

We define for a function $y \in \mathcal{C}$ the norm:

$$\|y(t)\|_r := \sup_{-r\le t\le 0} \|y(t)\|. \tag{13}$$

The proof that (13) is actually a norm on $\mathbb{R}^n$ is given in Driver (1977).

For an autonomous, constant delay problem the existence and uniqueness of solutions is assured by the following theorem (see Driver, 1977):

Theorem 1 (Global existence). *Let the functional $F : [t_0, t_f] \times \mathcal{C} \to \mathbb{R}^n$ be locally Lipschitz continuous. If further $F(t, y_t)$ satisfies the condition*:

$$\|F(t, y_t)\| \le M(t) + P(t)(\|y_t\|_r), \tag{14}$$

on $[t_0, t_f) \times \mathcal{C}$, where $M(t)$ and $P(t)$ are continuous positive definite functions for $t \in [t_0, t_f)$. Then the solution of the problem exists and is unique on the entire interval $[t_0 - r, t_f)$.

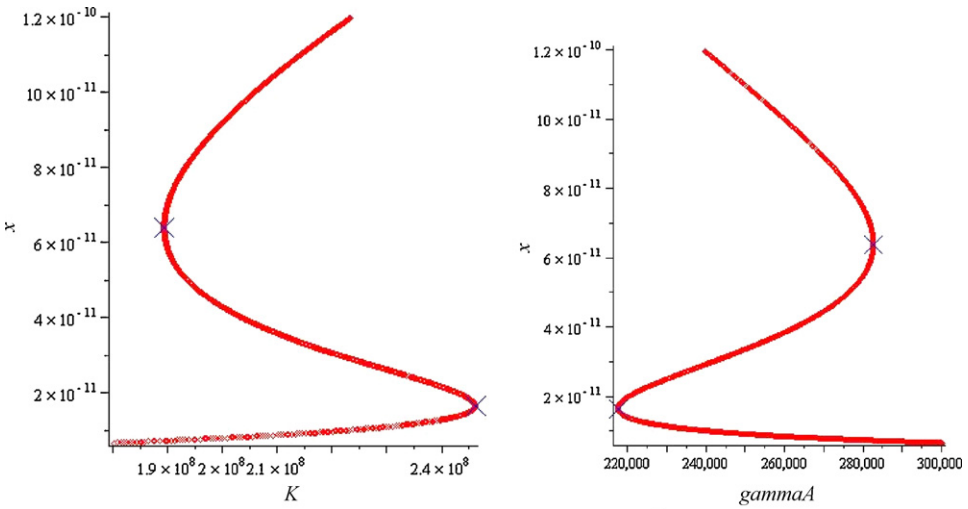

Fig. 4. Bifurcation diagrams for the steady states of 15: in (a) the bifurcation parameter is K, in (b) it is γ.

We invite the reader to notice that when speaking about the Lipschitz continuity of $F(t, y_t)$ with respect to y_t, we can also refer to the Lipschitz continuity of $f(t, u, v)$ with respect to u and v. Analoga to Theorem 1 are given in terms of Lipschitz continuity of $f(t, u, v)$ in Bellen and Zennaro (2003).

Since

$$\|u\| = |N| + |A| + |R_A| + |R| + |E|,$$

and

$$\|v\| = |N(t-\tau)| + |A(t-\tau)| + |R_A(t-\tau)| + |R(t-\tau)| + |E(t-\tau)| \geq |R_A(t-\tau)|,$$

one can easily verify that it holds:

$$\|f(t, u, v)\| \leq M(t) + P(t)(\|u\| + \|v\|),$$

where $M(t) := 0$ for all t in $[t_0, t_f)$ and $P(t)$ is a linear non-negative function (see Appendix A).

4.2. Bifurcations

In this Section we consider the dynamical system underlying the quorum sensing process described by our mathematical models. In order to investigate the long term dynamics, we first determine the stationary states (also called fixed points or equilibria) of the system and then look for possible bifurcations around them.

Given an autonomous dynamical system $\dot{x}(t) = f(x(t))$, with $x \in \mathbb{R}^n$ and $f : \mathbb{R}^n \to \mathbb{R}^n$, a stationary state $\bar{x}$ of the system is such that $f(\bar{x}) = 0$ (Driver, 1977; Guckenheimer and Holmes, 1990). This definition is actually given for ODEs, but as the reader will have noticed, the stationary states of a delay dynamical system $\dot{x}(t) = g(x(t), x(t-\tau))$ are the same as those of the corresponding non-delayed system ($\dot{x}(t) = g(x(t))$). Therefore it is enough for us to determine the fixed points of the system (1)–(5).

In order to investigate the existence and nature of stationary states, we reduce the dimension of the ODE system by applying some reasonable assumptions, without affecting the essential behaviour of the solutions. The bacterial population and the concentration of PpuR are assumed to be in a stationary state, i.e. the bacterial population has reached its capacity (K) and PpuR the "stationary condition" $\bar{R} = (\alpha_R/\gamma_R)\bar{N}$.

For the parameters, we assume $n_1 = n_2$ (denoted now just by n), $\gamma_3 = 0$ and $R_{A1} = R_{A2}$ (denoted by th in the following), since there is no biological argument against this assumption.

Consequently, (1)–(5) is reduced to the following two-dimensional system:

$$\begin{cases} \dot{A}(t) = \alpha K + \beta K \dfrac{R_A(t)^n}{th^n + R_A(t)^n} - \gamma A(t) - \alpha_1 \dfrac{\alpha_R}{\gamma_R} A(t) - K_E \dfrac{\alpha_E}{\gamma_E} K \dfrac{R_A(t)^n}{th^n + R_A(t)^n} A(t), \\ \dot{R}_A(t) = \alpha_1 \dfrac{\alpha_R}{\gamma_R} A(t) - \gamma_1 R_A(t). \end{cases}$$

The sought-after stationary conditions for the AHL concentration (and implicitly the stationary points for the whole system) can be found solving the following non-linear equation:

$$\beta K \frac{A(t)^n}{d^n + A(t)^n} - K K_E \frac{\alpha_E}{\gamma_E} \frac{A(t)^n}{d^n + A(t)^n} A(t) = \left(\gamma + \alpha_1 \frac{\alpha_R}{\gamma_R}\right) A(t) - \alpha K, \tag{15}$$

where $d := (\gamma_1 \gamma_R th)/(\alpha_1 \alpha_R)$.

Now we investigate the existence of possible bifurcation and choose the carrying capacity K and the abiotic degradation rate of AHLs γ as bifurcation parameters, since both may be perturbed externally.

First, we consider the bifurcation parameter K. A first saddle-node bifurcation happens at $K_A \approx 1.892 \times 10^8$ cells/l, a second saddle-node bifurcation appears at $K_B \approx 2.459 \times 10^8$ cells/l (see also Fig. 4(a)). That means that a small population cannot keep an induced AHL production, whereas a larger population will switch to the induced level in each case.

Similar holds for the abiotic degradation rate γ, which could also vary in a biological system, e.g. influenced by a change in the pH value (Englmann et al., 2007). When we choose γ as bifurcation parameter, we also find two saddle-node bifurcation points at $\gamma_A = 2.17e05$ 1/h and $\gamma_B = 2.82e05$ 1/h (see Fig. 4(b)). For the analysis of the stability of the points, we refer the reader to Barbarossa (2008).

Both for the bifurcation with respect to K and with respect to γ, we found a narrow interval of bistability, which may help to stabilize the system as a whole against perturbations.

4.3. Oscillations

The regulation system comprising the (delayed) lactonase activity, obviously contains a fast positive feedback and a slow negative feedback. In principle, such a structure allows for oscillations. The oscillating behaviour is particularly interesting since it allows to find a better approach for the first maximum reported in the experimental data.

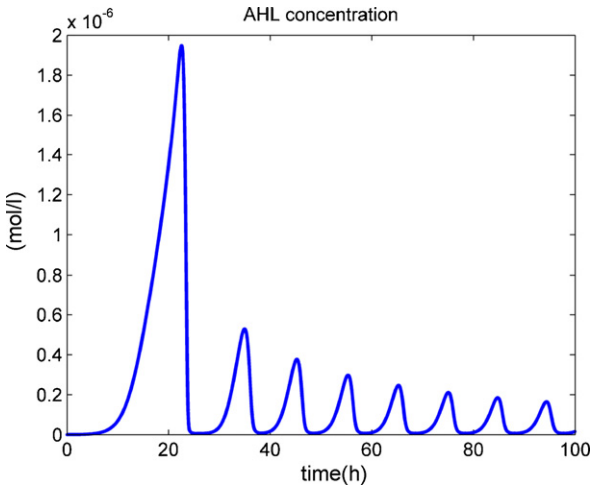

Fig. 5. Oscillation in the long-term dynamics of AHL.

For the biological meaning of the experiment, it is also interesting to investigate the long-term behaviour of the system. The solution was computed in the interval [0, 100] h and with particular history function and parameter values, an oscillating behaviour could be found (Fig. 5).

5. Discussion

The aim of this work was the qualitative description of the dynamics of the AHL production in the rhizosphere bacterium *P. putida* IsoF. As it has already been done for other bacterial species, e.g. *Pseudomonas aeruginosa* (Dockery and Keener, 2001) or *V. fischeri* (Kuttler and Hense, 2008), we first approached the problem by setting an ODE system.

In contrast with previous indirect measurements of the positive feedback, e.g. by luminescence (*V. fischeri*), the experimental data for *P. putida* IsoF showed a fast decline of the AHL concentration, short after the autoinduction had started. Further details, like the rapid decrease of AHL or the time course of the degradation products of AHL, lead to the assumption of the existence of a lactonase as AHL-degrading enzyme (Fekete et al., 2010).

In the present article, we analysed that model, which consists of a positive feedback loop (autoinduction of AHL production) and an additional negative feedback loop (AHL degradation by lactonase, also regulated by the PpuR-AHL complex). Such a combination allows for very different types of dynamic behaviour. The use of realistic parameter values permits the analysis of the regulation system for *P. putida* IsoF under realistic conditions. We could show, that also this system shows the typical bistable behaviour, choosing e.g. bacterial population density or abiotic degradation rate as exemplary bifurcation parameters. Even though the range of bistability is quite narrow, it allows to stabilize the system in its "on- or off-decision".

When considering the AHL concentrations during the first hours of the kinetic experiment, a first (smaller) temporal increase is measured in the baffled flasks and, to a much lower extent, also in the beaker experiment. Several repetitions of the experiment confirmed the presence of an initial peak in the AHL time series (data not shown). A possible explanation for this behaviour is a delay effect: from the preculture, there may still be an increased level of remaining PpuR-AHL complexes present in the cells when the bacteria are shifted to the new environment (beaker or baffled flasks). Thus, AHLs can be produced at a higher level at the beginning of the actual experiment, leading to the initial 'jump' in the AHL concentration in the first hours of the experiment. Because the AHLs transferred with the cells from the preculture are diluted 1000-fold in the fresh culture medium, the extracellular AHL concentration in the medium is expected to be initially very low, the same applies for the putative enzymatic degradation. Therefore, an initial high production could not be maintained because PpuR-AHL complexes are disappearing faster than they are formed, until the bacterial population size allows a sufficient accumulation of AHL in the medium, leading to the classical 'jump'. Using appropriate initial conditions for the model equations, we were able to show similar effects of transferring the bacteria from the preculture to the baffled flasks and the beaker. Probably, further effects from the transfer may influence the behaviour, which are not yet known in detail.

The introduction of a delay equation for the lactonase, taking into account that the production or activation of lactonase takes some time, yielded a much better quantitative (Fig. 3) accordance with the experiments than the original ODE model. From several spiking experiments (data not shown) we could estimate a delay of approximately 1–2 h for the lactonase activation. Thus, it is reasonable to make allowance for that delay. Smaller differences which still exist can be explained by further effects from the transfer of the bacteria which may influence their behaviour but which are not yet known in detail.

Altogether, the delay model justifies much better the existence of the two different maxima measured for the AHL concentration. This result was found by just introducing the delayed activity of the lactonase on the AHL molecules. The mathematical delay is supported by many biological hypotheses. It is very likely that regulation processes for the lactonase production retard the effect of this enzyme on the AHLs. Also the inoculation from the pre-culture to the flasks could delay certain activities of the bacteria (e.g. synthesis).

With a particular choice of the parameter values in the delay model, a possible oscillatory behaviour has been found (Fig. 5), which can be expected for this type of regulation system (combination of positive and negative feedback loop; Tyson and Othmer, 1978). One might ask, why these oscillations are not observed in our original system. For that, we should keep in mind, that we have to deal with a growing bacterial population. That is if the parameters are chosen in such a way, that the oscillations could appear in the case of a low population density, but not near the capacity of the system, we will not find them in the long time run.

Oscillations are actually the expected dynamics of an "ideal experiment" in which bacteria are always growing, without undergoing a stationary phase. These oscillations have not been observed yet experimentally. Possibly, the reason for that is that, under the given experimental conditions, the bacteria reach their stationary phase, which may lead to a change of metabolic behaviour; additionally, limited nutrients might play a role.

For a first investigation of the AHL dynamics in *P. putida* IsoF we did not insist on a better fitting of parameters, once the qualitative solution agreed with the experimental results. Of course, a better fitting of the parameter values is necessary for qualitative declarations, but this was not our major goal here.

Possible improvements of the model concern e.g. the choice of a different delay term for the lactonase equation. Indeed, instead of a constant delay one could choose a time-dependent delay, or a PpuR-AHL concentration-dependent delay. But as long as no further insights from a biological point of view are available for the explanation of the delay, we preferred to take the most simple, satisfactory assumption, to avoid too much arbitrariness in the model.

Appendix A.

Our delay model is essentially given by an ODE model (for which existence and uniqueness of solutions were assured) coupled with an autonomous constant delay equation. In order to have existence

and uniqueness of solutions (Theorem 1), we show that the functional $F(t, y_t)$ is local Lipschitz continuous with respect to y_t and that (14) holds.

We remind the reader that the parameter values present in the system are all equal or larger than zero, and that for biological reasons we are interested in non-negative solutions. The right hand side of system (1)–(4), (8) being defined in $\mathbb{R}^5_+$, we further assume $|\cdot|_r \equiv |\cdot|_1$. For improved readability, the time variable t will be omitted in the following (e.g. $N(t)$ will be indicated just by N).

The Lipschitz-continuity of $F(t, y_t)$ with respect to y_t is equivalent to the Lipschitz continuity of $f(t, u, v)$ with respect to u and v. In this case, the Lipschitz-continuity of $f(t, u, v)$ with respect to u is the same investigated for the ODE-system (see Barbarossa, 2008).

Before moving on with the investigation of the Lipschitz-continuity of $f(t, u, v)$ with respect to v, we give a look for boundedness of solutions of our system.

A.1. Boundedness of Solutions

We are interested in the boundedness of solutions of the system for future estimations on the partial derivatives of the right hand-side f.

Each component $y_i(t)$ of the system is indeed limited by a certain value $\hat{y}_i$, such that

$$y_i(t) > \hat{y}_i \Rightarrow \dot{y}_i(t) < 0.$$

We notice further that $A(t)$ is always non-negative, since the corresponding derivative $\dot{A}(t) > 0$ for $A(t) = 0$ and $A(0) \geq 0$. This holds for each component of the system, so we are sure that we deal with non-negative functions.

Let us start with the investigation of the bounds $\hat{y}_i$ for each component of the solution:

- The well-known logistic equation:

$$\dot{N}(t) = a\left(1 - \frac{N(t)}{K}\right)N(t)$$

has the solution

$$N(t) = \frac{N_0 K}{N_0 + (K - N_0)e^{-at}}. \tag{A.1}$$

For (A.1) it holds: $N_{\min} \leq N(t) \leq N_{\max}$ for all $t \geq 0$ where $N_{\max} := \max\{N_0, K\} < \infty$ and $N_{\min} := \min\{N_0, K\} > 0$.

- Let $\tilde{\gamma} := \min\{\gamma_1, \gamma_R\}$. Assuming

$$R_A(t) + R(t) > \frac{\alpha_R N_{\max}}{\tilde{\gamma}}$$

it follows that

$$(\dot{R}(t) + R_A(t)) = \alpha_R N(t) - \gamma_1 R_A(t) - \gamma_R R(t) \leq \alpha_R N_{\max} - \tilde{\gamma}(R_A(t) + R(t)) \leq 0$$

Define $R_{\max} := \max\{R_0 + R_{A0}, \frac{\alpha_R N_{\max}}{\tilde{\gamma}}\}$ and get the bounds: $0 \leq R(t) \leq R(t) + R_A(t) \leq R_{\max} < \infty$ for all $t \geq 0$ and analogously $0 \leq R_A(t) \leq R(t) + R_A(t) \leq R_{\max} < \infty$ for all $t \geq 0$.

- Now look for an upper-bound for $A(t)$:

$$A(t) > \frac{(\alpha+\beta)N_{\max} + \gamma_3 R_{\max}}{\gamma} \Rightarrow A(t) \geq \frac{(\alpha+\beta)N(t) + \gamma_3 R_A(t)}{\gamma}$$

With $A(t) > \frac{(\alpha+\beta)N_{\max}+\gamma_3 R_{\max}}{\gamma}$ it follows that:

$$\dot{A}(t) < -\left(\frac{(\alpha+\beta)N_{\max}+\gamma_3 R_{\max}}{\gamma}\right)\left(K_E E(t) + \alpha_1 \frac{R(t)}{N_{\min}}\right) \Rightarrow \dot{A}(t) < 0$$

and: $0 \leq A(t) \leq A_{\max} := \max\left\{A_0, \frac{(\alpha+\beta)N_{\max}+\gamma_3 R_{\max}}{\gamma}\right\} < \infty$ for all $t \geq 0$.

- Analogously, if

$$E(t) > \frac{\alpha N_{\max}}{\gamma_E} \Rightarrow E(t) \geq \frac{\alpha N(t)}{\gamma_E}$$

With $E(t) > \frac{\alpha N_{\max}}{\gamma_E}$ it follows that:

$$\dot{E}(t) < \alpha_E N(t) - \gamma_E \frac{\alpha N_{\max}}{\gamma_E} \Rightarrow \dot{E}(t) < 0$$

So it holds: $0 \leq E(t) \leq E_{\max} := \max\left\{E_0, \frac{\alpha N_{\max}}{\gamma_E}\right\} < \infty$ for all $t \geq 0$.

A.2. Existence and Uniqueness of Solutions

In this section we show that $f(t, u, v)$ is Lipschitz-continuous with respect to v and that Theorem 1 holds.

A sufficient condition for the (local) Lipschitz continuity of a function g is the (local) C^1-continuity together with the boundedness of all its partial derivatives $\frac{\partial g_i}{\partial x_j}$. The proof of the boundedness of the partial derivatives of $f(t, u, v)$ with respect to u is given for the interested reader in section A.3.

In our case, all partial derivatives of f with respect to the deviated argument $v := u(t - \tau)$ are zero apart from $\frac{\partial f_5}{\partial v_3} = \frac{\partial \dot{E}}{\partial R_A}$:

$$\frac{\partial f_5}{\partial v_3} = \alpha_E \frac{n_2 R_{A2}^{n_2} R_A(t-\tau)^{n_2-1}}{(R_{A2}^{n_2} + R_A(t-\tau)^{n_2})^2} \tag{A.2}$$

Function (A.2) is continuous in $\mathbb{R}_+$. Considering the computed estimations of the solutions and the corresponding notation, we find:

$$\left|\frac{\partial f_5}{\partial v_3}\right| \leq \alpha_E N_{\max} n_2 \frac{R_{\max}^{n_2-1}}{R_{A2}^{n_2}}$$

Since all parameter values are in $[0, \infty)$, the derivative is bounded and we have proved the Lipschitz continuity of f with respect to the deviated argument v.

According to Theorem 1, we have to demonstrate

$$\|f(t, u, v)\| \leq M(t) + P(t)(\|u\| + \|v\|)$$

to have global existence and uniqueness of solutions.

Let $|\cdot| = |\cdot|_1 = |f_1| + \cdots + |f_5|$ and proceed with the estimation of each single component of this sum.

We begin with the boundedness of the first component of the f:

$$|f_1| \leq a\left(1 + \frac{N_{\max}}{N_{\min}}\right)|N|. \tag{A.3}$$

For the second component of f it holds:

$$|f_2| \leq (\alpha+\beta)|N| + \gamma|A| + K_E|E||A| + \frac{\alpha_1}{N_{\min}}|A||R| + \gamma_3|R_A| \leq (\alpha+\beta)|N| + \gamma|A| + K_E A_{\max}|E| + \frac{\alpha_1 A_{\max}|R|}{N_{\min}} + \gamma_3|R_A|. \tag{A.4}$$

An upper bound for the third component of f is given by:

$$|f_3| \leq \frac{\alpha_1}{N_{\min}} A_{\max}|R| + (\gamma_1 + \gamma_3)|R_A|. \tag{A.5}$$

Analogously,

$$|f_4| \leq \alpha_R|N| + \frac{\alpha_1}{N_{\min}} A_{\max}|R| + \gamma_3|R_A| + \gamma_R|R|. \tag{A.6}$$

For the fifth component of the right hand-side it holds:

$$|f_5| \leq \frac{\alpha_E N_{\max}}{R_{A2}^{n_2}} R_{\max}^{(n_2-1)} |R_A(t-\tau)| + \gamma_E |E|. \tag{A.7}$$

Thanks to the upper-bounds given by (A.3)–(A.7), it holds:

$$\begin{aligned}\|f\| &= |f_1| + |f_2| + |f_3| + |f_4| + |f_5| \leq a\left(1 + \frac{N_{\max}}{N_{\min}}\right)|N| + (\alpha+\beta)|N| \\ &+\gamma|A| + K_E A_{\max}|E| + \frac{\alpha_1}{N_{\min}} A_{\max}|R| + \gamma_3|R_A| + \frac{\alpha_1}{N_{\min}} A_{\max}|R| \\ &+(\gamma_1+\gamma_3)|R_A| + \alpha_R|N| + \frac{\alpha_1}{N_{\min}} A_{\max}|R| + \gamma_3|R_A| + \gamma_R|R| \\ &+\frac{\alpha_E N_{\max}}{R_{A2}^{n_2}} R_{\max}^{(n_2-1)}|R_A(t-\tau)| + \gamma_E|E|. \end{aligned} \tag{A.8}$$

We define

$$P(t) := \max\left\{\frac{\alpha_1}{N_{\min}} A_{\max} + \gamma_R,\ 2a + \alpha + \beta + \alpha_R,\ \gamma,\ 3\gamma_3 + \gamma_1,\ \gamma_E,\ \frac{\alpha_E N_{\max}}{R_{A2}^{n_2}} R_{\max}^{(n_2-1)}\right\} \tag{A.9}$$

and $M(t) := 0$ for all t in $[t_0, t_f)$.

Considering further that

$$\|u\| = |N| + |A| + |R_A| + |R| + |E|$$

and that

$$\begin{aligned}\|v\| &= |N(t-\tau)| + |A(t-\tau)| + |R_A(t-\tau)| + |R(t-\tau)| + |E(t-\tau)| \\ &\geq |R_A(t-\tau)|\end{aligned}$$

we have verified that

$$\|f(t,u,v)\| \leq M(t) + P(t)(\|u\| + \|v\|).$$

The requested assumptions are proved and Theorem 1 holds. The existence and uniqueness of solutions of the delay problem (1)–(4), (8) is assured in $[t_0, t_f)$.

A.3. Boundedness of the Partial Derivatives

In the following we show that $f(t,u,v)$ is Lipschitz-continuous with respect to u. The bounds $N_{\max}, \ldots, E_{\max}$ we found in section A.1 will simplify the estimation of the modulus of the partial derivatives of the right-hand side f of the DDE problem. We denote f_i the ith component of f and u_j the jth component of the solution (e.g. $f_1 \equiv \dot{N}$, $u_3 \equiv R_A$) and omit the time variable t.

- For f_1 it holds:

$$\left|\frac{\partial f_1}{\partial u_1}\right| \leq a + 2a\frac{N_{\max}}{N_{\min}}$$

and $\left|\frac{\partial f_1}{\partial u_i}\right| = 0$, for $i = 2,\ 3,\ 4,\ 5$.

- The partial derivatives of f_2 are bounded as follows:

$$\begin{aligned}\left|\frac{\partial f_2}{\partial u_1}\right| &\leq \alpha + \beta + \frac{\alpha_1 R_{\max} A_{\max}}{N_{\min}^2} \\ \left|\frac{\partial f_2}{\partial u_2}\right| &\leq \gamma + \frac{\alpha_1 R_{\max}}{N_{\min}} + K_E E_{\max} \\ \left|\frac{\partial f_2}{\partial u_3}\right| &\leq \beta N_{\max} \frac{n_1 R_{\max}^{n_1-1}}{R_{A1}^{n_1}} + \gamma_3 \\ \left|\frac{\partial f_2}{\partial u_4}\right| &\leq \alpha_1 \frac{A_{\max}}{N_{\min}} \\ \left|\frac{\partial f_2}{\partial u_5}\right| &\leq \alpha_E A_{\max}\end{aligned}$$

- The partial derivatives of f_3 are bounded as follows:

$$\begin{aligned}\left|\frac{\partial f_3}{\partial u_1}\right| &\leq \frac{\alpha_1 R_{\max} A_{\max}}{N_{\min}^2} \\ \left|\frac{\partial f_3}{\partial u_2}\right| &\leq \frac{\alpha_1 R_{\max}}{N_{\min}} \\ \left|\frac{\partial f_3}{\partial u_3}\right| &\leq \gamma_1 + \gamma_3 \\ \left|\frac{\partial f_3}{\partial u_4}\right| &\leq \alpha_1 \frac{A_{\max}}{N_{\min}} \\ \left|\frac{\partial f_3}{\partial u_5}\right| &= 0\end{aligned}$$

- Analogously for the partial derivatives of f_4:

$$\begin{aligned}\left|\frac{\partial f_4}{\partial u_1}\right| &\leq \alpha_R + \frac{\alpha_1 R_{\max} A_{\max}}{N_{\min}^2} \\ \left|\frac{\partial f_4}{\partial u_2}\right| &\leq \frac{\alpha_1 R_{\max}}{N_{\min}} \\ \left|\frac{\partial f_4}{\partial u_3}\right| &\leq \gamma_3 \\ \left|\frac{\partial f_4}{\partial u_4}\right| &\leq \alpha_1 \frac{A_{\max}}{N_{\min}} + \gamma_R \\ \left|\frac{\partial f_4}{\partial u_5}\right| &= 0\end{aligned}$$

- For the partial derivatives of the last component of f, it holds:

$$\begin{aligned}\left|\frac{\partial f_5}{\partial u_1}\right| &\leq \alpha_E \\ \left|\frac{\partial f_5}{\partial u_2}\right| &= 0 \\ \left|\frac{\partial f_5}{\partial u_3}\right| &= 0 \\ \left|\frac{\partial f_5}{\partial u_4}\right| &= 0 \\ \left|\frac{\partial f_5}{\partial u_5}\right| &\leq \gamma_E\end{aligned}$$

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