We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

7

Oscillators for Modelling Circadian Rhythms in Cyanobacteria Growth

Jaromír Fišer[a], Jan Červený[b] and Pavel Zítek[a]
[a] *Czech Technical University in Prague, Centre for Applied Cybernetics (cAk) Czech Republic*
[b] *Institute of Systems Biology and Ecology in Nové Hrady, Academy of Sciences CR Czech Republic*

1. Introduction

There is strong evidence that the behaviour of living systems is subject to biological clocks which can be considered as mutually coupled oscillators. These applications of oscillators were studied since very early (Minorsky, 1962; Pavlidis, 1973). In case of biological systems like algae populations or micro-organism cultures their varying growth rate and other living system activities are liable, first of all, to the diurnal cycles of light irradiation as the decisive model input. The living systems adopt these cyclic conditions as their inner circadian rhythms and exert a specific tendency to maintain their rhythm even if the cyclic external influences change their period or shape. In this way the model of system entrainment to circadian rhythms is based on the idea of nonlinear resonance phenomenon. The circadian rhythms, also referred to as internal biological rhythms, play a role as temporal regulatory pacemakers practically in any activity of living species, but their mechanism remains still largely unknown (Ditty et al., 2009). Experimental studies and mathematical modelling have demonstrated that circadian pacemakers working on periods close to 24 hours can be modelled as limit cycle oscillators (Pavlidis, 1973; Winfree, 1970; Wever, 1970). Typically a pacemaker model implementation involves a Van der Pol oscillator as a limit cycle generator influencing the model of population growth (Fišer et al., 2008). Then this circadian pacemaker structure can be identified with the experimentally obtained data. A part of the recent research in cyanobacteria growth modelling has been already described in the previous paper (Fišer et al., 2006), where an algae population growth is investigated.

In selecting a suitable oscillator for circadian pacemaker application the ability of the system to adapt its frequency and the shape of cycles according to the exogenous cyclic inputs, is to be kept in view in particular. Thus any oscillator considered as the pacemaker has to be endowed with the property that its limit cycle oscillations change gradually in frequency and shape according to the cyclic influences. These influences comprise light irradiance and other ambient inputs particularly temperature and nourishment supply (Johnson et al., 2004).

Beside the analytical nonlinear schemes mentioned above a chemical oscillator has already been developed as generator of circadian rhythms (Miyoshi et al., 2007), and its operation was tested on rhythms in cyanobacteria. The structure of this oscillator is relatively complex and the aim of this chapter is to find an approximation of the Miyoshi oscillator by a Van der Pol type oscillator for substituting its function by a simpler scheme in modelling the timing influence on the diurnal cycles in the cyanobacteria growth.

2. Experimental data acquisition

The authors' own data material used for working out the model presented below, originates from experiments with unicellular, diazotropic cyanobacterium *Cyanothece,* sp. ATCC 51142. The experiments were performed in a laboratory-scale bioreactor, developed by Photon Systems Instruments, Ltd. (Fig.1). Due to a programmable source of variable irradiance in this device and due to other adjustable experiment conditions artificially formed diurnal cycles can be provided and the consequent circadian rhythms in cyanobacteria culture can be observed and recorded. The used bioreactor provides capability to generate artificially defined cultivation conditions with controlled distribution of CO_2 nourishment, inevitable for obtaining consistent culture growth data. Furthermore, a controlled heating and/or cooling allows to maintaining a desired temperature for cultivation. Continuously recorded outputs include temperature, optical density and fluorescence emission representing the culture production performance. Among other artificial perturbations of the culture growth changes of the nourishing gas composition can be provided. Particularly the carbon dioxide concentration changes can be applied in the experiments. As the model output the optical density of cyanobacteria culture in 735 nm, as a parameter proportional to concentration of cyanobacteria culture, is used (Nedbal et al., 2008).

Fig. 1. Prototype of cultivation device

3. Population growth model

Among other approaches to modelling the population growth, the models based on Volterra population equation are applied. Their recent applications used to include the delay effects resulting from the age structure of the described population into the model (Cushing, 1993; Iannelli, 1994). The delayed Volterra model of population growth is usually referred to in a functional form which can be found in Kuang, (1993). To express the ageing influence on the inhibition of population growth an extension of the delayed Volterra model has been introduced by Fišer et al., (2007).

Consider the population growth model described by the delayed Volterra-type system (Červený et al., 2007)

$$\frac{dx(t)}{dt} = \left(\mu(x, y, I, \vartheta) - \int_0^T x(t-\tau) dA(\tau) - \int_0^T \frac{dx(t-\tau)}{dt} dB(\tau) \right) x(t) \tag{1}$$

where x is the cyanobacteria concentration in the culture, I is the incident light intensity, ϑ is the temperature of nourishing medium and $\mu(x, y, I, \vartheta)$ is the specific rate of cell growth. This growth rate is also affected by the timing activity of the biological clock of the culture represented by the cyclic variable y as the output of oscillator described below. The functions $A(\tau) > 0$, $B(\tau) > 0$ are delay distributions, $\tau \geq 0$, and T is the maximum delay length. As to the timing action its impact on the specific growth rate is considered in the following separated form

$$\mu(x, y, I, \vartheta) = \mu_p(x, I, \vartheta) + \mu_c(y) \tag{2}$$

where μ_p expresses the specific growth rate based on Monod kinetics and μ_c is a growth rate increment originating from the clock oscillator. The variable y is the output of a chemical cyclic action explained in Section 4 which controls the circadian rhythms. Due to the dimensional homogeneity its influence on the growth rate is supposed as proportional to one of the state variable derivatives of the oscillator in Section 4

$$\mu_c(t) = C\, y(t) = C \frac{d[CPKaiC_6]}{dt} \tag{3}$$

where C is a proportional gain coefficient.

The value of the main component μ_p of the growth rate results from both the Monod kinetics and Lambert-Beer law as follows

$$\mu(x, I, \vartheta) = \mu_{\max} \left(1 - e^{-\frac{I_a(x,I)}{I_{sat}}} \right) p(\vartheta) \tag{4}$$

where I_a is the average light intensity inside the culture given by

$$I_a(x,I) = \frac{I\,k_w}{k_c x}\left[1 - e^{-k_c x}\right] \tag{5}$$

where I_{sat} is a saturation light intensity, and p is an auxiliary function expressing the dependance of the growth rate on the temperature. The parameters k_c, k_w determine the light absorption in cyanobacteria culture and glass wall, respectively (for more details see Li et al., 2003).

In the next the main attention is paid to circadian rhythm issue, i.e. the cyclic influence of $\mu_c(y)$ on the cyanobacteria growth rate.

4. Miyoshi chemical oscillator

The functional structure of circadian oscillator in cyanobacteria on molecular level was discovered by Ishiura et al. (1998). The experiment data were measured on *Cyanothece* sp. while one has to be concerned about already published models which are almost exclusively developed for another model cyanobacterium *Synechococcus elongatus* (e.g. Miyoshi et al., 2007; Mori et al., 2007; Rust et al., 2007; van Zon et al., 2007). For our modelling approach let be assumed that the features of interest in these two organisms are comparable. More details on molecular base of cyanobacteria circadian clock are well described recently by Ditty et al. (2009).

For purposes of diurnal cyanobacteria growth modelling we adopted the oscillator developed by Miyoshi et al. (2007) that allows entrainment by the light-dark forcing applied on the culture. Adopted mechanistic model of oscillator constitutes the set of 13 differential equations that describe changes in concentration of protein complexes involved in circadian clock system. The equations with state variable descriptions (Table 1) are as follows

$$\frac{d[KaiC_6]}{dt} = -[KaiC_6]\left(k_1 + k_{21} + v_{cat1}[KaiC_6]^{-1}\right) + k_{22}[PPKaiC_6] + k_2[KaiC]^6 + v_{cat2} \tag{6}$$

$$\frac{d[PPKaiC_6]}{dt} = -[PPKaiC_6]\left(k_3 + k_{22} + k_{23}\right) + k_4[KaiC]^3[PKaiC]^3 + k_{21}[KaiC_6] + k_{24}[CPKaiC_6] + v_{cat1} - v_{cat2} - v_{cat3} + v_{cat4} \tag{7}$$

$$\frac{d[CPKaiC_6]}{dt} = y = -[CPKaiC_6]\left(k_5 + k_{24}\right) + k_6[PKaiC]^6 + k_{23}[PPKaiC_6] + v_{cat3} - v_{cat4} \tag{8}$$

$$\frac{d[KaiC]}{dt} = 6\left(k_1[KaiC_6] - k_2[KaiC]^6\right) + 3\left(k_3[PPKaiC_6] - k_4[KaiC]^3[PKaiC]^3\right) + \left(k_{tl2}[kaiBC\,mRNA] - k_{p\deg 3}[KaiC]\right)L \tag{9}$$

State variables	Description	Init. cond. [molecules/cell]
$[KaiC_6]$	non-phosphorylated KaiC hexameric complex	139.220
$[PPKaiC_6]$	partially phosphorylated KaiC hexameric complex	779.158
$[CPKaiC_6]$	completely phosphorylated KaiC hexameric complex	1229.563
$[KaiC]$	non-phosphorylated KaiC monomer	932.446
$[PKaiC]$	phosphorylated KaiC monomer	110.829
$[KaiB_4]$	KaiB-inactive tetramer complex	0.173
$[KaiB_{4i}]$	KaiB-active tetramer complex	3251.369
$[KaiB]$	KaiB monomer	1130
$[KaiA_2]$	KaiA dimer	166.559
$[KaiA]$	KaiA monomer	9.998
$[kaiA\ mRNA]$	kaiA mRNA	2.856
$[kaiBC\ mRNA]$	kaiBC mRNA	2.865
$[KaiA_2B_4]$	complex of a KaiA dimer and KaiB-active tetramer	51.022

Table 1. State variables description with initial conditions

$$\frac{d[PKaiC]}{dt} = -[PKaiC]^3 3\left(k_4[KaiC]^3 + 2k_6[PKaiC]^3\right) + 3k_3[PPKaiC_6] + 6k_5[CPKaiC_6] - k_{p\deg 4}[PKaiC]L \tag{10}$$

$$\frac{d[KaiB_4]}{dt} = -[KaiB_4]\left(k_{10} + k_{11}[KaiA_2]\right) + k_9[KaiB]^4 + k_{12}[KaiA_2B_4] - v_{cat\_b1} + v_{cat\_b2} \tag{11}$$

$$\frac{d[KaiB_{4i}]}{dt} = v_{cat\_b1} - v_{cat\_b2} \tag{12}$$

$$\frac{d[KaiB]}{dt} = 4\left(k_{10}[KaiB_4] - k_9[KaiB]^4\right) + \left(k_{tl2}[kaiBC\ mRNA] - k_{p\deg 2}[KaiB]\right)L \tag{13}$$

$$\frac{d[KaiA_2]}{dt} = -[KaiA_2]\left(k_7 + k_{11}[KaiB_4]\right) + k_8[KaiA]^2 + k_{12}[KaiA_2B_4] \tag{14}$$

$$\frac{d[KaiA]}{dt} = -[KaiA]\left(2k_8[KaiA] + k_{p\deg 1}L\right) + 2k_7[KaiA_2] + k_{tl1}[kaiA\ mRNA]L \tag{15}$$

$$\frac{d[kaiA\ mRNA]}{dt} = k_{a1}\frac{k_{bts1}[RNAP]}{1 + k_{bts1}[RNAP]}\frac{[CPKaiC_6]}{[PPKaiC_6]}L - k_{m\deg 1}[kaiA\ mRNA] \tag{16}$$

$$\frac{d[kaiBC\ mRNA]}{dt} = k_{a2}\frac{k_{bts2}[RNAP]}{1+k_{bts2}[RNAP]}\frac{[CPKaiC_6]}{[PPKaiC_6]}L - k_{m\deg 2}[kaiBC\ mRNA] \tag{17}$$

$$\frac{d[KaiA_2B_4]}{dt} = k_{11}[KaiA_2][KaiB_4] - k_{12}[KaiA_2B_4] \tag{18}$$

where rate variables formed from Michaelis-Menten equations are as follows

$$v_{cat1} = k_{cat1}\frac{[KaiA_2][KaiC_6]}{K_{m1}+[KaiC_6]} \tag{19}$$

$$v_{cat2} = k_{cat2}\frac{[KaiA_2B_4][PPKaiC_6]}{K_{m2}+[PPKaiC_6]} \tag{20}$$

$$v_{cat3} = k_{cat3}\frac{[KaiA_2][PPKaiC_6]}{K_{m3}+[PPKaiC_6]} \tag{21}$$

$$v_{cat4} = k_{cat4}\frac{[KaiA_2B_4][CPKaiC_6]}{K_{m4}+[CPKaiC_6]} \tag{22}$$

$$v_{cat\_b1} = k_{cat\_b1}\frac{[PPKaiC_6][KaiB_4]}{K_{m\_b1}+[KaiB_4]} \tag{23}$$

$$v_{cat\_b2} = k_{cat\_b2}\frac{[CPKaiC_6][KaiB_{4i}]}{K_{m\_b2}+[KaiB_{4i}]} \tag{24}$$

The parameter values and variable description, of all the rate constants starting in the notation with k and all the Michaelis constants starting in the notation with K are specified in the application example. Also the initial conditions of the set (6)-(18) are provided in the application example. Because some rate variables in (6)-(18) are activated by forcing light (for original reference see Miyoshi et al., 2007), while in dark these rate variables are relaxed, we apply to distinguish between the activation and relaxation of these rate variables a logical variable L already substituted into (6)-(18). This logical variable represents on/off irradiance state (for more details see Section 5).

For the simulation purposes (6)-(18) are viewed as the state equations in state variables specified by Table 1 and constituting the state vector as follows

$$\mathbf{w} = \begin{bmatrix} [KaiC_6],[PPKaiC_6],[CPKaiC_6],[KaiC],[PKaiC],[KaiB_4],[KaiB_{4i}],... \\ ...[KaiB],[KaiA_2],[KaiA],[kaiA\ mRNA],[kaiBC\ mRNA],[KaiA_2B_4] \end{bmatrix}^T \tag{25}$$

Correcting several misprints in Miyoshi et al. (2007) the following changes were carried out in (11) and (13). Namely, the signs of rate variables $v_9 = k_9[KaiB]^4$, $v_{10} = k_{10}[KaiB_4]$ are exchanged, and at the same time the right-hand sides of expressions for v_9, v_{10} are mutually exchanged. Another correction was applied to parameters k_{cat4} and $k_{p\deg 4}$. In addition, parameters K_{m1}, K_{m2} were increased 1000 times to achieve the circadian rhythms of 24 h period.

5. Approximation of biological clock by Van der Pol oscillator

An oscillator based on Van der Pol equation is proposed to approximate the chemical oscillator presented in previous section because of appreciable simplification concerning the number of both state variables and tuning parameters. Then, let the proposed Van der Pol oscillator be considered in the matrix form

$$\frac{d\mathbf{z}(t)}{dt} = \mathbf{A}\mathbf{z}(t) + \mathbf{F}(\mathbf{z})\mathbf{z}(t)z_1(t) + \mathbf{B}(\mathbf{z})\mathbf{u}(t,D) \tag{26}$$

where $\mathbf{z} = [z_1, z_2, z_3]^T$ is the state vector of the oscillator. Both the state and input matrices are

$$\mathbf{A} = \begin{bmatrix} 0 & 1 & 0 \\ 0 & a\varepsilon & 0 \\ 0 & 0 & -\frac{1}{T} \end{bmatrix}, \ \mathbf{F}(\mathbf{z}) = \begin{bmatrix} 0 & 0 & 0 \\ -abz_2 & 0 & -1 \\ 0 & 0 & -\frac{1}{T} \end{bmatrix} \tag{27}$$

and

$$\mathbf{B}(\mathbf{z}) = \begin{bmatrix} 0 & \frac{q}{4\pi^2} z_3 & 0 \\ 0 & 0 & \frac{\Omega}{T} \end{bmatrix}^T \tag{28}$$

respectively. The constants a, ε, b are the parameters of Van der Pol equation and the oscillator input vector is of the form $\mathbf{u}(t,D) = [I(t) \quad \Omega(t-D)]^T$, where $I(t)$ is the cyclic light intensity representing the diurnal cycles, and Ω is the frequency on which the oscillator is to be entrained. Gain q amplifies the light intensity and time constant T with pure delay D determines the dynamics of adaptation. The existence of limit cycle motion is conditioned by the inequality

$$\left(\varepsilon - bz_1^2\right) > 0 \tag{29}$$

where $\varepsilon > 0$, $b > 0$. The other parameters, a and q, are the weighting coefficients which influence the shape of limit cycle oscillations. The oscillator output is given by the equation

$$y(t) = C_d z_2(t) = C_d \frac{dz_1(t)}{dt} \tag{30}$$

where C_d is a normalization coefficient. Output y is then the input variable of specific growth rate (3) to modify the cyanobacteria growth by circadian rhythms as $\mu_c(t) = C\, y(t) = C\, C_d\, z_2(t)$.

6. Oscillator-based scheme for adapting to circadian rhythms

Basically equation (1) provides the model with the internal relationships which govern the growth of population but this model part does not express the circadian character and particularly the impact of diurnal cycles on the growth. The biological populations are specifically sensitive to changes in their environment and thus they are able to adapt themselves to these changes. All this suggests that a more complex mechanism than a pure sensory adaptation may be involved in the model. It is typical that the adaptation time constants tend to be rather longer than the ones typical for sensoric organ responses. This property of biological systems is provided by means of applying an oscillator (Pavlidis, 1973) influenced by a generator of diurnal cycles as in the block diagram in Fig. 2. Using the oscillator based on Van der Pol equation, the generator of diurnal cycles of light irradiation *I*(*t*) may be applied to adapt the limit cycle frequency of the oscillator. Another oscillator, called chemical, is forced by sequence of 0 and 1, where 0 and 1 correspond to the dark phase and light phase, respectively. This is simply done using logical variable *L*, see Fig. 2, that either 0 or 1 are in the product with corresponding rate variables in right-hand sides of (8)-(20). We say that these rate variables are in on/off irradiance state.

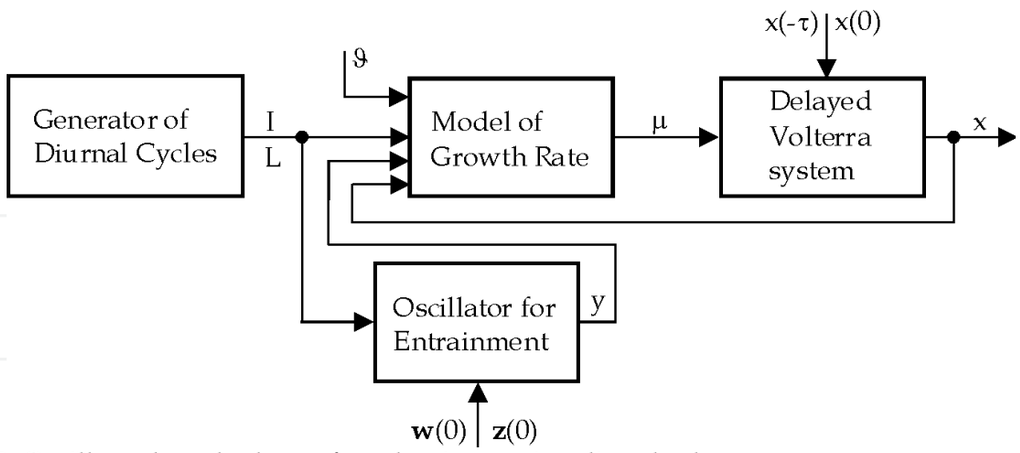


Fig. 2. Oscillator-based scheme for adapting to circadian rhythms

The modelling with the help of the scheme in Fig. 2 requires the setting of proper initial conditions comprised in vectors $\mathbf{w}(0)$, $\mathbf{z}(0)$ and initial cyanobacteria concentration (inoculum) $x(0)$ with relaxed $x(-\tau), 0 < \tau \le \tau_{\max}$.

7. Application example

In this section a growth model for cyanobacteria species *Cyanothece* is presented. For fitting the model the data from experiments reported in Section 2 were used. The samples of measured courses of cyanobacteria, like that in Fig. 5 were used to identify the model parameters. First, the parameters of specific growth rate μ were determined, for more details we refer to Červený et al., (2007), where the cultivated cyanobacteria species *Cyanothece* is investigated. In this paper the following parameters are considered: $\mu_{\max} = 0.028\,h^{-1}$, $I_{sat} = 126\,\mathrm{Wm}^{-2}$, auxiliary value $p(\vartheta = 30°C) = 1$, coefficient $k_c = 3.4$, specific parameter $k_w = 31.6$. The remaining parameters of model (1) have been resulted in the distributions $A(\tau)$ and $B(\tau)$ as follows

$$A(\tau) = \begin{cases} 0, & \tau < 0 \\ 3.33 \cdot \dfrac{\tau}{24}, & \tau \in \langle 0,24 \rangle \text{ h}, \; B(\tau) = 0 \\ 3.33, & \tau > 24 \end{cases} \tag{31}$$

First, the parameters of chemical oscillator are determined in the table below (adopted from Supplementary Online Material in Červený & Nedbal (2009)).

Rate constants	*Description*
$k_1 = 1.615\,h^{-1}$	dissociation rate for $KaiC_6$
$k_2 = 2.039 \times 10^{-16}\,molecules^{-5}\,cell^{5}\,h^{-1}$	binding rate for KaiC
$k_3 = 1.615 \times 10^{-4}\,h^{-1}$	dissociation rate for $PPKaiC_6$
$k_4 = 1.019 \times 10^{-14}\,molecules^{-5}\,cell^{5}\,h^{-1}$	binding rate for KaiC and PKaiC
$k_5 = 0.162\,h^{-1}$	dissociation rate for $CPKaiC_6$
$k_6 = 1.019 \times 10^{-10}\,molecules^{-5}\,cell^{5}\,h^{-1}$	binding constant for PKaiC
$k_7 = 0.162 \times 10^{-1}\,h^{-1}$	dissociation rate for $KaiA_2$
$k_8 = 0.268\,molecules^{-1}\,cell^{1}\,h^{-1}$	binding rate for KaiA
$k_9 = 7.393 \times 10^{-17}\,molecules^{-3}\,cell^{3}\,h^{-1}$	binding rate for KaiB
$k_{10} = 1.615 \times 10^{-4}\,h^{-1}$	dissociation rate for $KaiB_4$
$k_{11} = 8.756 \times 10^{-4}\,molecules^{-1}\,cell^{1}\,h^{-1}$	binding rate for $KaiA_2$ and $KaiB_4$
$k_{12} = 8.788 \times 10^{-2}\,h^{-1}$	dissociation rate for $KaiA_2$
$k_{21} = 1.079 \times 10^{-8}\,h^{-1}$	autophosphorylation rate of $KaiA_2B_4$
$k_{22} = 1.079 \times 10^{-5}\,h^{-1}$	autodephosphorylation rate of $PPKaiC_6$
$k_{23} = 1.079 \times 10^{-6}\,h^{-1}$	autophosphorylation rate of $PPKaiC_6$
$k_{24} = 1.079 \times 10^{-8}\,h^{-1}$	autodephosphorylation rate of $CPKaiC_6$
$k_{a1} = 1.017 \times 10^{7}\,molecules\,cell^{-1}\,h^{-1}$	transcription rate of kaiA

$k_{a2} = 6.458 \times 10^{7}\ molecules\ cell^{-1} h^{-1}$	transcription rate of kaiBC
$k_{cat1} = 0.539\ h^{-1}$	rate of $KaiC_6$ phosphorylation
$k_{cat2} = 0.539\ h^{-1}$	rate of $PPKaiC_6$ dephosphorylation
$k_{cat3} = 1.079\ h^{-1}$	rate of $PPKaiC_6$ phosphorylation
$k_{cat4} = 0.890\ h^{-1}$	rate of $CPKaiC_6$ dephosphorylation
$k_{cat\_b1} = 2.423\ h^{-1}$	rate of $KaiB_4$ inactivation
$k_{cat\_b2} = 0.346\ h^{-1}$	rate of $KaiB_{4i}$ activation
$k_{m\deg 1} = 0.133\ h^{-1}$	degradation rate of kaiA mRNA
$k_{m\deg 2} = 0.178\ h^{-1}$	degradation rate of kaiBC mRNA
$k_{p\deg 1} = 8.00 \times 10^{-3}\ h^{-1}$	degradation rate of KaiA
$k_{p\deg 2} = 0.490\ h^{-1}$	degradation rate of KaiB
$k_{p\deg 3} = 1.300\ h^{-1}$	degradation rate of KaiC
$k_{p\deg 4} = 0.200\ h^{-1}$	degradation rate of PKaiC
$k_{tl1} = 8.239 \times 10^{-3}\ h^{-1}$	translation rate of kaiA
$k_{tl2} = 1.701 \times 10^{2}\ h^{-1}$	translation rate of kaiBC
Michaelis and miscellaneous constants	*Description*
$k_{bts1} = 3.657 \times 10^{-12}\ molecules^{-1}\ cell$	binding constant for RNA polymerase in *kaiA*
$k_{bts1} = 1.000 \times 10^{-12}\ molecules^{-1}\ cell$	binding constant for RNA polymerase in *kaiBC*
$K_{m1} = 602\ molecules\ cell^{-1}$	Michaelis constant for $KaiC_6$ phosphorylation
$K_{m2} = 602\ molecules\ cell^{-1}$	Michaelis constant for $PPKaiC_6$ dephosphorylation
$K_{m3} = 0.602\ molecules\ cell^{-1}$	Michaelis constant for $PPKaiC_6$ phosphorylation
$K_{m4} = 0.602\ molecules\ cell^{-1}$	Michaelis constant for $CPKaiB_4$ dephosphorylation
$K_{m\_b1} = 0.602\ molecules\ cell^{-1}$	Michaelis constant for $KaiB_4$ inactivation
$K_{m\_b2} = 6.675 \times 10^{1}\ molecules\ cell^{-1}$	Michaelis constant for $KaiB_{4i}$ activation
$[RNAP] = 5000\ molecules\ cell^{-1}$	RNA polymerase concentration

Table 2. Rate and other constants in equations (6) – (24)

Then the parameters of Van der Pol oscillator are found as follows: $\Omega = 0.26\ \mathrm{rad} \cdot \mathrm{h}^{-1}\ (2\pi/24)$, $\varepsilon = 1$, $b = 181$, $a = 0.03$, $q = 0.03$, $T = 1\ \mathrm{h}$ and $D = 0$. Proportional gain C results in $5 \times 10^{-4}\ \mathrm{molecules}^{-1} \cdot \mathrm{cell}$ for the chemical oscillator and $2\ \mathrm{molecules}^{-1} \cdot \mathrm{cell}$ for the Van der Pol oscillator. In addition, normalization coefficient C_d in (30) is adjusted at the value $2\ \mathrm{molecules} \cdot \mathrm{cell}^{-1}$. For comparing both oscillators the initial conditions of the chemical

oscillator are set in their "morning" values, introduced by Miyoshi et al. (2007), are listed in Table 1. Obviously, the third –order oscillator of Van der Pol type cannot satisfy these conditions but its limit cycle can be satisfactorily identified with that of the chemical oscillator. In order to set Van der Pol oscillator output close to limit cycle the following initial conditions are used

$$z_1(0) = -0.085, z_2(0) = 0, z_3(0) = \Omega^2 \tag{32}$$

where Ω is the frequency of desired circadian rhythm.

The initial conditions of the chemical oscillator are chosen to be synchronized with cyanobacteria circadian oscillator. In Fig. 3 the phase portraits of the chemical and Van der Pol oscillator are recorded.

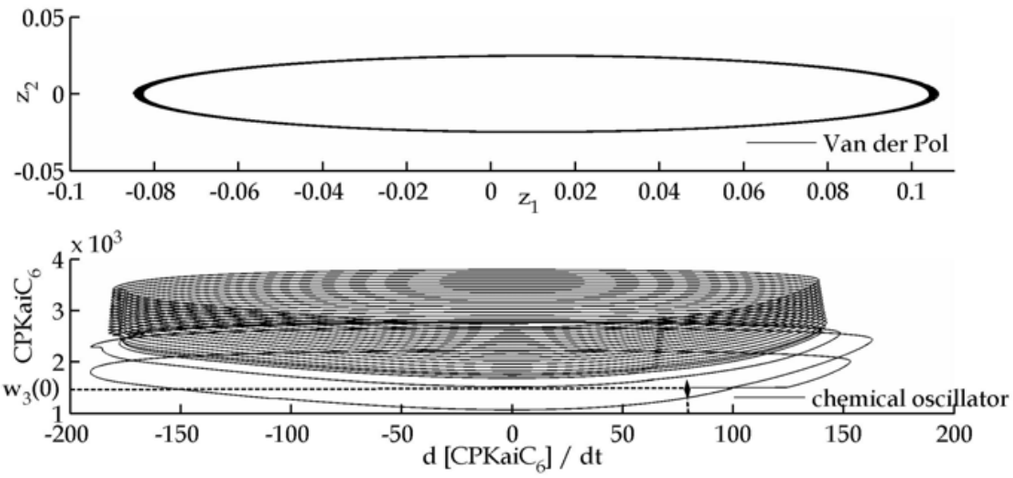


Fig. 3. Phase portraits of both oscillators with initial conditions in Table 1 and (32)

After comparing limit cycles of both oscillators the chemical oscillator tends to the limit cycle along a spiral while the Van der Pol oscillator immediately achieves the limit cycle. However, the subject of interest is to apply the variables on horizontal axes in Fig. 3 what are the derivatives changing the specific growth rate in (1).

The specific growth rate given by (2), composed from two components, is drawn in Fig. 4 under light conditions specified in Fig. 5.

In Fig. 5 the cyanobacteria growth is obtained in circadian LD 12:12 regime, where LD regime abbreviates light/dark regime in hours. Later on, the LD regime is switched to LL regime where LL denotes continuous light.

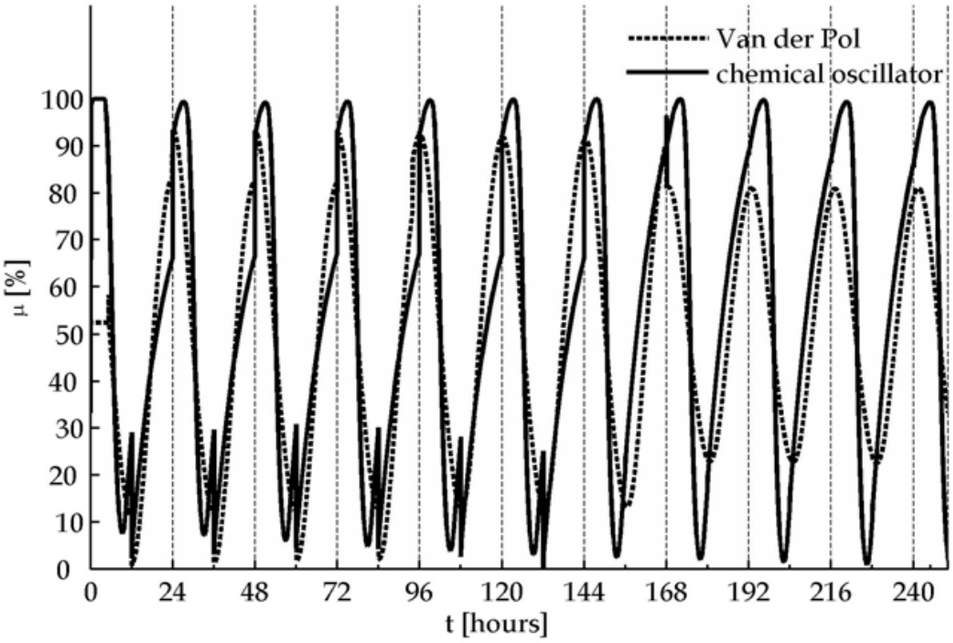


Fig. 4. Specific growth rate (2) in percents with respect to $\mu_{\max}$

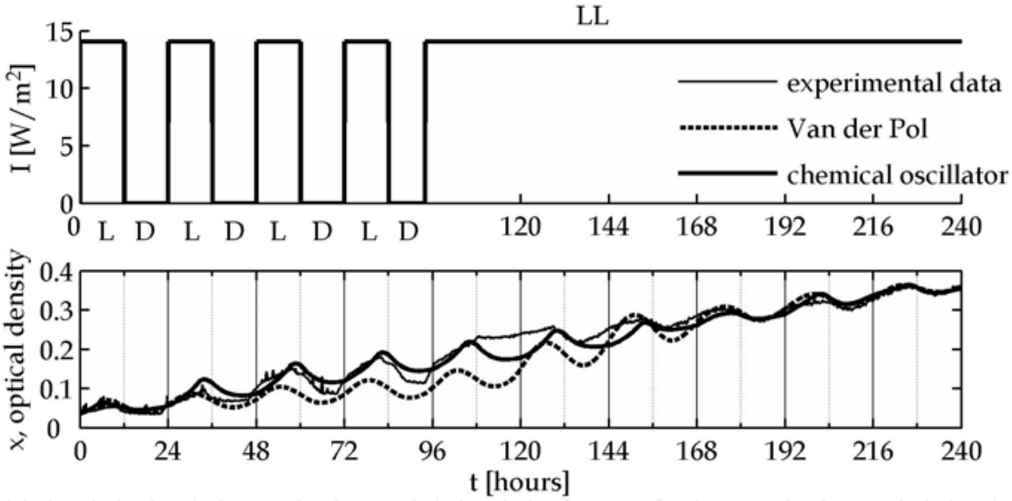


Fig. 5. Comparison of measured concentration x with the modelled one using the scheme in Fig. 2 with initial conditions $x(0) = 0.045, x(-\tau) = 0, \quad 0 < \tau \leq 24\text{h}$

In Fig. 5 an experiment with cyanobacteria culture is recorded, where the cyclic lighting with 24 hours period is changed to a permanent light with constant intensity. The measured data of cyanobacteria growth are compared with the modelling results obtained from both the models with chemical and Van der Pol oscillators. Both the models fit well the undisturbed growth of the culture, however, the model version with the chemical oscillator is in a better agreement with the experiments, better than it is with the application of Van der Pol oscillator.

8. Conclusions

The issue of circadian rhythms is getting more importance with the emerging possibility of intensifying the biotechnological processes. The main aim of the paper is to show that the rather complex chemical oscillator can be substituted by a relatively simple Van der Pol oscillator in its timing function in modelling the circadian rhythms. While the chemical oscillator consists of thirteen state variables the simple Van der Pol is of the third order only. Apparently these oscillators cannot be fully equivalent in their state vectors, but both the oscillators can substitute each other in generating the clock limit cycle. The basic frequency is given by the 24 hour period and both these oscillators can be tuned to a different desired frequency. Nevertheless the adjustment of the period is not of the same dynamics in both the oscillators. Only in this respect the Van der Pol oscillator does not fit the growth oscillations in cyanobacteria as the chemical one, but this shortage is not substantial for the bioreactor application and is outweighed by the simplicity of the proposed approximation. As regards the presented results it is necessary to note that the experiment conditions were simplified as to the simplified nourishment technique, where only CO_2 was supplied while no fixable nitrogen was available. That is why in the presented experiments the cyanobacteria concentration drops during the dark phases, which is not typical in case of complete nourishment.

Acknowledgement

This work was supported by the Ministry of Education of the Czech Republic under Project 1M0567, and subsequently by grants AV0Z60870520 (Czech Academy of Sciences) and GACR 206/09/1284 (Czech Science Foundation) and through the FUNCDYN Programme of the European Science Foundation and European Commission, Contract no. ERAS-CT-2003-980409.

9. References

Cushing, J. M. (1993). An Introduction to Structured Population Dynamics, In: *Regional Conference Series in Applied Mathematics (No. 71),* Rozier, R., (Ed.), pp. 200, SIAM, ISBN 0-89871-417-6, Philadelphia

Červený, J.; Fišer, J. & Zítek P. (2007). A Model Solution to Adapting the Algae Population Growth to Diurnal Cycles, *Proceedings of 16th Int. Conf. Process Control (PC'07)*, ISBN 978-80-227-2677-1, Štrbské Pleso, High Tatras, Slovakia, 11-14 June 2007, STU, Bratislava

Červený, J.; Nedbal, L. (2009). Metabolic rhythms of the cyanobacterium *Cyanothece* sp. ATCC 51142 correlate with modeled dynamics of circadian clock, *Journal of Biological Rhythms*, Vol. 24, No. 4, (Aug 2009), pp. 295-303, ISSN 0748-7304

Ditty, J. L.; Mackey, S. R. & Johnson, C. H. (2009). *Bacterial Circadian Programs*, Springer, ISBN 978-3-540-88430-9, Berlin

Fišer, J.; Červený, J. & Zítek P. (2006). Time Delay Model of Algal Population Growth in a Photobioreactor, *Proceedings of 5th MathMod*, Vol. 2, ISBN 3-901608-30-3, Vienna, Austria, 8-10 February 2006, Argesim, Wien

Fišer, J.; Červený, J. & Zítek P. (2007). Time Delay Model of Algal Population Growth in Tubular Photobioreactor, *SNE(Simulation News Europe),* Vol. 17, No. 3-4, (Dec 2007), 14-18, ISSN 0929-2268

Fišer, J.; Zítek P. & Červený, J. (2008). Oscillators for Modeling Biomass Growth Adaptation to Circadian Rhythms, *Proceedings of UKSIM Tenth International Conference on Computer Modelling and Simulation*, pp. 175-179, ISBN 0-7695-3114-8, Cambridge, UK, 1-3 April 2008, IEEE Computer Society, Los Alamitos

Iannelli, M. (1994). Mathematical Theory of Age-structured Population Dynamics, In: *Applied Mathematics Monographs*, Vol. 7, Pasquali, A., (Ed.), pp. 174, Giardini Editori e Stampatori, ISBN 88-427-0250-1, Pisa

Ishiura, M.; Kutsuna, S.; Aoki, S.; Iwasaki, H.; Andersson, C. R.; Tanabe, A.; Golden, S. S.; Johnson, C. H. & Kondo, T. (1998). Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria. *Science*, Vol. 281, No. 5382, (Sep 1998), 1519-1523, ISSN 0036-8075

Johnson, C. H.; Elliott, J.; Foster, R.; Honma, K-I. & Kronauer, R. (2004). Fundamental properties of circadian rhythms, In: *Chronobiology: Biological Timekeeping*, Dunlap, J. C.; Loros, J. J. & DeCoursey, P. J. (Ed.), pp. 66-105, Sinauer Associates, Inc. Publishers, ISBN 0-87893-149-X, Sunderland

Kuang, Y. (1993). Delay Differential Equations, with Applications in Population Dynamics. In: *Mathematics in Science and Eng.*, Vol. 191, Ames, W. F., (Ed.), pp. 400, Academic Press, ISBN 0-12-427610-5, San Diego

Li, J.; Xu, N. S. & Su, W. W. (2003). Online estimation of stirred-tank microalgal photobioreactor cultures based on dissolved oxygen measurement, *Biochemical Engineering Journal*, Vol. 14, No. 1, (April 2003), 51-65, ISSN 1369-703X

Minorsky, N. (1962). Nonlinear Oscillations, Van Nostrand Company, Inc., ISBN 0-442-05408-4, Princeton, New Jersey

Miyoshi, F.; Nakayama, Y.; Kaizu, K.; Iwasaki, H. & Tomita M. (2007). A Mathematical Model for the Kai-Protein-Based Chemical Oscillator and Clock Gene Expression Rhythms in Cyanobacteria, *Journal of Biological Rhythms*, Vol. 22, No. 1, (Feb 2007), 69-80, ISSN 0748-7304

Mori, T.; Williams, D. R.; Byrne, M. O.; Qin, X.; Egli, M.; McHaourab, H. S.; Stewart, P. L. & Johnson, C. H. (2007). Elucidating the ticking of an *in vitro* circadian clockwork. *PLoS Biology*, Vol. 5, No. 4, (March 2007), e93, ISSN 1544-9173, doi:10.1371/journal.pbio.0050093

Nedbal, L.; Trtílek, M.; Červený, J.; Komárek, O. & Pakrasi H. B. (2008). A Photobioreactor System for Precision Cultivation of Photoautotrophic Microorganisms and for High-Content Analysis of Suspension Dynamics, *Biotechnology and Bioengineering*, Vol. 100, No. 5, (Aug 2008), 902-910, ISSN 0006-3592

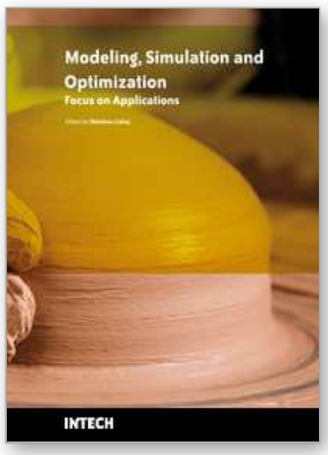
Pavlidis, T. (1973). *Biological Oscillators: Their Mathematical Analysis,* Academic Press, ISBN 0-12-547350-8, New York

Rust, M. J.; Markson, J. S.; Lane, W. S.; Fisher, D. S. & O'Shea, E. K. (2007). Ordered phosphorylation governs oscillation of a three-protein circadian clock. *Science*, Vol. 318, No. 5851, 809-812, ISSN 0036-8075

van Zon, J. S.; Lubensky, D. K.; Altena, P. R. H. & ten Wolde P. R (2007). An allosteric model of circadian KaiC phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 104, No. 18, (April 2007), 7420-7425, ISSN 0027-8424

Wever, R. (1987). Mathematical Models of Circadian One- and Multi-Oscillator Systems, In: *Some Mathematical Questions in Biology: Circadian Rhythms,* Carpenter, G. A., (Ed.), pp. 205-265, Lectures on Mathematics in the Life Sciences, Vol. 19, American Mathematical Society, ISBN 0-8218-1169-X, Providence

Winfree, A. T. (1970). Integrated View Resetting a Circadian Clock, *Journal of Theoretical Biology,* Vol. 28, No. 3, (Sept 1970), 327-335, ISSN 0022-5193



Modeling Simulation and Optimization - Focus on Applications

Edited by Shkelzen Cakaj

ISBN 978-953-307-055-1
Hard cover, 312 pages
Publisher InTech
Published online 01, March, 2010
Published in print edition March, 2010

The book presents a collection of chapters dealing with a wide selection of topics concerning different applications of modeling. It includes modeling, simulation and optimization applications in the areas of medical care systems, genetics, business, ethics and linguistics, applying very sophisticated methods. Algorithms, 3-D modeling, virtual reality, multi objective optimization, finite element methods, multi agent model simulation, system dynamics simulation, hierarchical Petri Net model and two level formalism modeling are tools and methods employed in these papers.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen