Synchronization of Metabolic Oscillations: Two Cells and Ensembles of Adsorbed Cells

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Abstract. We treat synchronization of metabolic oscillations in two cells and in ensembles of cells adsorbed at the liquid-solid interface. (i) Synchronization of oscillations in two cells is assumed to occur via perturbation of the metabolite concentration near one cell due to the metabolite diffusion flux from another cell. This *direct* channel of synchronization may be important if the distance between two cells is comparable with the cell diameter. The corresponding coupling coefficient is found to be proportional to the metabolite diffusion coefficient and inversely proportional to the cell radius and the distance between the cells. (ii) In the case of ensembles of adsorbed cells, synchronization of oscillations is considered to be *indirect*, i.e., to occur via the metabolite concentration formed outside the cells near the interface due to metabolite diffusion from the cells. We have derived a general integral equation relating the metabolite concentration near the interface with concentrations inside the cells.

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1. Introduction

Kinetic equations describing chemical reactions are usually nonlinear. Under certain conditions, the nonlinearities may result in kinetic oscillations, chaos and pattern formations. Such effects have been observed in many reactions occurring in gas phase, liquid and on solid surfaces [1–3]. In biochemical cellular reactions, kinetic oscillations have been observed on the size scales ranging from macroscopic, in cell extracts and suspensions, to mesoscopic in individual cells (see the monograph by Goldbeter [4], recent experiments [5–7], and references therein). Observations of oscillations in cell suspensions on the macroscopic scale indicate that there exist cell-cell communication resulting in synchronization of individual oscillators. One of the ways to study synchronization of cellular oscillations is to mix two suspensions of cells that oscillate with equal amplitude and opposite phases. In such experiments, oscillations first almost disappear, but then again develop and reach an asymptotic amplitude (see, e.g., recent studies [5] of glycolytic oscillations in

yeast cells). Physically, synchronization of oscillations in cells occurs via reversible diffusion of one of the metabolites out of cells. In yeast glycolytic oscillations, for example, such a metabolite is found [6] to be acetaldehyde. Cells at different phases share the extracellular acetaldehyde concentration. This interconnection between cells results asymptotically in synchronization of oscillations.

Mathematical approaches aimed at describing synchronization of oscillations in cells are diverse. Historically, the first approach was proposed by Prigogine and Lefever [8] in order to treat phenomenologically the interplay of two cells or two equivalent ensembles of cells (provided that the reaction kinetics inside each ensemble are synchronized). The corresponding equations for two two-variable chemical oscillators are as follows (for a recent example of application of such equations, see [5]).

$$\begin{aligned} dx_1/dt &= f(x_1, y_1), \\ dy_1/dt &= g(x_1, y_1) + v(y_2 - y_1), \\ dx_2/dt &= f(x_2, y_2), \\ dy_2/dt &= g(x_2, y_2) + v(y_1 - y_2), \end{aligned}$$

where x_1, y_1, x_2 and y_2 are the metabolite concentrations, $f(x, y)$ and $g(x, y)$ are the functions defining the dynamics of a single oscillator, and v is the coupling coefficient.

The equations above do not describe explicitly the metabolites outside the cells. To incorporate the latter feature into simulations, Romanivskii et al. [9] proposed to complement the equations for single oscillators by that for a 'bath' formed by the extracellular metabolite. In particular, two equivalent two-variable ensembles of oscillators are treated as follows (for application of this approach, see e.g. [10])

$$\begin{aligned} dx_1/dt &= f(x_1, y_1), \\ dy_1/dt &= g(x_1, y_1) + v(y_e - y_1), \\ dx_2/dt &= f(x_2, y_2), \\ dy_2/dt &= g(x_2, y_2) + v(y_e - y_2), \\ dy_e/dt &= w(y_1 + y_2 - 2y_e) - \gamma y_e, \end{aligned}$$

where y_e is the extracellular concentration, v and w are the coupling coefficients, and γ is the rate constant of the metabolite removal due to reaction(s) outside the cells.

The equations above are applicable provided that the metabolite diffusion inside and outside the cells is relatively rapid (no phase delay). A more general treatment of the diffusion-mediated coupling of chemical oscillators includes integral equations for reactant concentrations [11]. There are also a few more formal approaches to the problem under consideration, e.g., those based on the phenomenological equations for the oscillator phases [12] or on coupled logistic maps [13].

The general understanding of the behaviour of coupled oscillators is relatively complete. Frequently, the coupling of two harmonic oscillators leads to 'in phase' synchronization of sustained oscillations. However, one can also observe termination of oscillations or period doubling, tripling and/or chaos. On a more detailed

level, the field is still far from exhausted, and many interesting questions remain open.

One such question concerns the coefficients that couple the concentrations of metabolites belonging to different cells. In the equations above and other phenomenological approaches, these coefficients are postulated. This makes sense if one is interested in synchronization of ensembles of cells in the case when it occurs indirectly, i.e., through one of the metabolites distributed uniformly outside the cells in the extracellular space (which we call the 'bath'). In this practically important case, the coupling coefficients characterize the cell-bath communication and can be treated as fitting parameters. Direct communication between cells is more intriguing, because in the latter case the coupling coefficient should be dependent on the distance between the cells. The latter case has not been analyzed in detail, because such communication was negligibly weak in the experiments done so far (note that the metabolic oscillations are usually studied in the bulk of solution under conditions where the average distance between cells is much larger than the cell size).

Our present paper is focused primarily on direct communication between cells. To further motivate our study, we note that in metabolic oscillations due to direct cell-cell communication are soon likely to be experimentally explored in the case when cells are adsorbed at the liquid-solid interface. Already now, numerous studies are ongoing with cell cultures on biochemically functional surfaces, in such a way that they will allow real time and spatially resolved observations of cell-cell communication [14]. The driving force behind this is both a basic research interests and numerous applications, e.g., tissue engineering, cell sensors and bioelectronics. Employing, e.g., electron-beam lithography [15, 16], one can fabricate specially designed heterogeneous surfaces containing μm spots or other chemical/topographic patterns for cell adsorption. The size of such spots, the distance between them, and their relative arrangement can easily be varied. With this approach, one can, for example, prepare pairs of spots with a prescribed, desirable distance between their centers, adsorb cells on these spots, and then explore communicative metabolic oscillations in the cells. Finally, we note that cell in real tissue are often closely packed and may communicate over distances comparable to or less than the cell size.

Motivated by the above points, we analyze (Sec. 2) communication between two cells separated at a distance comparable with the cell diameter. In addition, we derive and briefly discuss (Sec. 3) the equations describing indirect (through the bath) communication in an ensemble of adsorbed cells.

Figure 1. Two cells located (a) in the bulk of solution and (b) at the liquid-solid interface.

2. Direct Communication between Two Cells

2.1. MODEL

To motivate our analysis, we noted in the Introduction that direct communication between cells is expected to be important first of all in the case of adsorbed cells. Formally, however, the results obtained for surface immobilized cells are directly applicable to cells located in the bulk of solution. For this reason, we treat both cases simultaneously. Specifically, the cells are considered to be spherical [Figure 1(a)], if they are located in the bulk of solution, and hemispherical [Figure 1(b)], if they are adsorbed. Communication between cells is assumed to occur via reversible diffusion of one of the metabolites out of or into cells.

The details of the treatment of the problem under consideration depend on the ratio between the period of metabolic oscillations inside a cell, τ_{osc} , and the time

scales, characterizing respectively the metabolite diffusion inside and between the cells, $\tau_{\text{int}} = \rho^2/D$ and $\tau_{\text{ext}} = l^2/D$ (D is here the diffusion coefficient, ρ the cell radius, and l the distance between the cells). In the present study, we analyze one of the practically important situations when the metabolite diffusion is rapid, i.e.,

$$\tau_{\text{osc}}/\tau_{\text{int}} \equiv \tau_{\text{osc}}D/\rho^2 \gg 1, \quad (1)$$

$$\tau_{\text{osc}}/\tau_{\text{ext}} \equiv \tau_{\text{osc}}D/l^2 \gg 1, \quad (2)$$

and

$$\gamma\tau_{\text{ext}} \equiv \gamma l^2/D \ll 1, \quad (3)$$

where γ is the rate constant of the metabolite removal outside the cells. Specifically, condition (1) is assumed to hold for all the metabolites inside the cells. In this case, the gradients of the metabolite concentrations inside the cells are negligible.

To calculate the coupling between the cells, we need to describe the metabolite that is able to diffuse out of cells. For this metabolite, the concentrations inside and outside the two cells are designated as $n_1(t)$, $n_2(t)$, and $n(\mathbf{r}, t)$, respectively. If the metabolite diffusion outside the cells is fast [Eq. (2)] and the metabolite drain due to reaction outside the cells is nearly negligible [Eq. (3)], the concentration $n(\mathbf{r}, t)$ can be calculated by solving the steady-state equation for the metabolite diffusion,

$$\nabla^2 n(\mathbf{r}) = 0. \quad (4)$$

Note that in the steady-state approximation, there is no need to indicate time explicitly. For this reason, we use here $n(\mathbf{r})$ instead of $n(\mathbf{r}, t)$ and below n_1 and n_2 instead of $n_1(t)$ and $n_2(t)$.

The boundary conditions for Eq. (4) depend on the mechanism of the metabolite transport through the cell membranes. In our study, the metabolite diffusion via the membranes is assumed to be passive or facilitated by membrane enzymes. The membranes are considered to be homogeneous. If diffusion occurs via the membrane enzymes, the enzyme distribution in the membranes is assumed to be homogeneous as well. In addition, the metabolite charge is considered to be zero, and accordingly the effect of the membrane electric field on diffusion is neglected. Under such circumstances, the boundary conditions near the cell membranes are as follows

$$k(n_1 - n(\mathbf{r})) = -D \frac{\partial n(\mathbf{r})}{\partial r} \quad \text{for } \mathbf{r} \in \mathbf{s}_1, \quad (5)$$

$$k(n_2 - n(\mathbf{r})) = -D \frac{\partial n(\mathbf{r})}{\partial r} \quad \text{for } \mathbf{r} \in \mathbf{s}_2, \quad (6)$$

where k is the rate constant corresponding to the metabolite transport from one side of a membrane to another side, $\partial n/\partial r$ is the partial derivatives calculated along the normal to the membranes, and $\mathbf{s}_1$ and $\mathbf{s}_2$ are the symbols defining the location of

the membranes. Physically, the boundary conditions (5) and (6) indicate that the diffusion flux through the cell membranes should be equal to the diffusion flux just outside the cells.

In the case of adsorbed cells [Figure 1(b)], we assume that the metabolite does not interact with the interface (no adsorption). In this case, the boundary condition near the interface is given by

$$\frac{\partial n(\mathbf{r})}{\partial r} = 0 \quad \text{for } \mathbf{r} \in \mathbf{s}, \quad (7)$$

where $\partial n/\partial r$ is the partial derivatives calculated along the normal, and $\mathbf{s}$ is the symbol defining the location of the interface.

For cells in the bulk [Figure 1(a)], the solution of Eq. (4) with the boundary conditions (5) and (6) possesses cylindrical symmetry with respect to the line connecting the cells, and accordingly condition (7) holds automatically. For this reason, the results obtained below are equally applicable to cells located in the bulk of solution and at the interface.

The total fluxes of the metabolite out of the cells are defined by integration of the diffusion fluxes over the membrane areas,

$$J_1 = -D \oint \frac{\partial n(\mathbf{r})}{\partial r} ds_1 \quad (8)$$

and

$$J_2 = -D \oint \frac{\partial n(\mathbf{r})}{\partial r} ds_2. \quad (9)$$

For adjacent cells (Figure 1), the fluxes J_1 and J_2 depend on the metabolite concentrations in both cells, i.e., on n_1 and n_2 . This interplay of the fluxes results in cell-cell communication. In particular, the fluxes J_1 and J_2 can be used in the mean-field equations for n_1 and n_2 . Analyzing the dependence of J_1 and J_2 on n_1 and n_2 , one can obtain the coupling coefficients for these concentrations. Thus, Eqs. (4–9) form a mathematical basis for our model.

2.2. COUPLING COEFFICIENTS

To calculate the coupling coefficients, we need to solve Eq. (4) with the corresponding boundary conditions in the case when the distance between two cells is comparable with the cell radius. First, it is instructive however to recall the conventional solution to Eq. (4) in the limit $l \rightarrow \infty$. In this limit, the communication between cells is negligible, i.e., calculating $n(r)$ near one cell, we may ignore any other cell. In particular, the metabolite distribution near the first cell is given by

$$n(r) = c\rho n_1/r, \quad (10)$$

where r is the radial coordinate, and c is a dimensionless constant defined as

$$c = k\rho/(k\rho + D). \quad (11)$$

For the total diffusion flux from a spherical cell, we accordingly have

$$J_1 = 4\pi c\rho Dn_1. \quad (12)$$

The total diffusion flux from a hemispherical cell is two times smaller. The flux per unit volume of the cell, j_1 , is obtained by dividing J_1 by the cell volume [$V = (4/3)\pi\rho^3$ or $V = (2/3)\pi\rho^3$], i.e.,

$$j_1 = J_1/V = k_d n_1, \quad (13)$$

where

$$k_d = 3cD/\rho^2 \quad (14)$$

is the rate constant corresponding to the metabolite diffusion out of the cell. By its definition, this rate constant can be directly employed in the kinetic equation for n_1 ,

$$dn_1/dt = g(n_1) - k_d n_1, \quad (15)$$

where $g(n_1)$ is the function determining the metabolite dynamics in the case when diffusion out of the cell is negligible (this function actually depends not only on n_1 but also on the other metabolite concentrations).

In the case of two adjacent cells (Figure 1), the solution to Eq. (4) can be constructed by using the Legendre polynomials. To obtain compact results, we employ the first two polynomials and perturbation theory in order to find the corresponding expansion coefficients. This approach makes it possible to obtain the first term of expansion of the coupling coefficients in series over $1/l$. Specifically, the metabolite concentration is represented as superposition of four terms satisfying Eq. (4),

$$n(\mathbf{r}) = \frac{c_1}{r} + \frac{c_2(\mathbf{r}\mathbf{e})}{r^3} + \frac{c_3}{|\mathbf{r} - \mathbf{l}|} + \frac{c_4((\mathbf{r} - \mathbf{l})\mathbf{e})}{|\mathbf{r} - \mathbf{l}|^3}, \quad (16)$$

where c_i are the expansion coefficients, $\mathbf{e}$ is the unit vector directed from one cell to another, and $\mathbf{l}$ is the vector connecting the cell centers. Thus, to solve our problem, we only need to choose the expansion coefficients in agreement with the boundary conditions (5) and (6).

Taking into account that the perturbation of the diffusion flux from one cell by another cell is weak, we use the solution obtained for a single cell [Eqs. (10) and (11)] as the zero-order approximation for two cells, i.e.,

$$c_1^{(0)} = c\rho n_1, \quad c_3^{(0)} = c\rho n_2, \quad \text{and} \quad c_2^{(0)} = c_4^{(0)} = 0. \quad (17)$$

The zero-order perturbation of the metabolite concentration near the first cell by the diffusion flux from the second cell can then be expanded as

$$\frac{c_3^{(0)}}{|\mathbf{r} - \mathbf{l}|} \simeq \frac{c_3^{(0)}}{l} + \frac{c_3^{(0)}(\mathbf{r}\mathbf{e})}{l^2}. \quad (18)$$

To satisfy, with this perturbation, the boundary condition (5) near the first cell, we should introduce the first-order corrections to the coefficients c_1 and c_2 . In particular, the perturbation of the boundary condition (5) by the second term in the right-hand part of Eq. (18) can be compensated by the first-order correction of the coefficient c_2 . These corrections are however not of interest, because due to their symmetry they do not contribute to the total diffusion flux from the first cell. What we really need in order to calculate the latter flux is the first-order correction to the coefficient c_1 . Representing this coefficient as $c_1 = c_1^{(0)} + c_1^{(1)}$, taking into account the first term in the right-hand part of Eq. (18), and using the boundary condition (5), we obtain

$$c_1^{(1)} \simeq -\frac{c^2 \rho^2}{l} n_2. \quad (19)$$

The corresponding correction of the diffusion flux from the first cell is given by (provided that the cell is spherical)

$$J_1^{(1)} = -\frac{4\pi c^2 \rho^2 D}{l} n_2. \quad (20)$$

For hemispherical cells, the corresponding correction is two times smaller. Dividing these corrections by V yields the corresponding correction of the flux per unit volume

$$j_1^{(1)} = -\frac{3c^2 D}{\rho l} n_2. \quad (21)$$

Employing the latter expression, we can represent the kinetic equation for n_1 as

$$dn_1/dt = g(n_1) - k_d n_1 + v n_2, \quad (22)$$

where k_d is the rate constant defined by Eq. (14), and

$$v = \frac{3c^2 D}{\rho l} \quad (23)$$

is the coupling coefficient. For the second cell, we obtain in analogy

$$dn_2/dt = g(n_2) - k_d n_2 + v n_1. \quad (24)$$

Expression (23) indicates that the coupling coefficient is proportional to the diffusion coefficient and inversely proportional to the cell radius and the distance between the cells. In addition, it is of interest to notice that the rate constants k_d and v are interconnected [cf. Eqs. (14) and (23)] as $v = (c\rho/l)k_d$. Our analysis, based on the perturbation theory, is valid provided that $(c\rho/l) \ll 1$.

Finally, noting that $k_d \propto c$ and $v \propto c^2$, it makes sense to discuss the coefficient c in more detail. According to Eq. (11), this coefficient depends on the ratio between $k\rho$ and D . To specify k , one has in general to develop a microscopic theory. The

physics behind the rate constant k can however be understood by using the simplest phenomenological effective-medium model. According to this model, we have

$$k \simeq \phi \exp(-\Delta E/k_B T) D_m / h, \quad (25)$$

where ϕ is the ratio of the membrane surface providing channels for the metabolite transport to the whole membrane surface ($\phi = 1$ and $\ll 1$ for passive or enzyme-facilitated diffusion, respectively), $\Delta E > 0$ the metabolite energy difference in the membrane and solution, D_m the effective coefficient for diffusion in the membrane, and h the membrane width. Taking into account expression (25), we expect that the ratio $k\rho/D$ may often be much lower than unity, because $\phi \leq 1$, $D_m \leq D$ and $\Delta E \geq 0$ (note, however, that $\rho/h \gg 1$). If this is the case, the constant c [Eq. (11)] is much smaller than unity as well.

2.3. EXAMPLE OF SYNCHRONIZATION OF OSCILLATIONS

Our analysis above indicates that the kinetic equations describing diffusion-mediated communication between two cells [see, e.g., Eqs. (22) and (24)] contain two types of interconnected terms. The terms of the first type [e.g., $k_d n_1$ in Eq. (22)], taking into account the metabolite release from a cell, result in reduction of the metabolite concentration inside the cell. The terms of the second type [e.g., vn_2 in Eq. (22)] are connected with perturbation of the metabolite concentration near a cell due to the metabolite flux from another cell. The latter terms, responsible for cell-cell communication, result in increase of the metabolite concentration in a cell. In this subsection, we demonstrate how these terms may affect the oscillatory kinetics in two cells. Specifically, we complement one of the models describing oscillations inside cells by the terms obtained in our study and show the corresponding kinetics.

For example, we employ a simple two-variable model used recently by Bier et al. [5] for describing synchronization of glycolytic oscillations in yeast cells. It includes autocatalytic conversion of substrate to product,

and irreversible removal of the product,

where A is the metabolite non-participating in oscillations. In reality, steps (26) and (27) run via substeps including enzymes. To simplify the analysis, the rate of reaction (26) is considered to be proportional to the product of S and P concentrations. Reaction (27) is assumed to occur on enzymes via the conventional Michaelis-Menten mechanism.

Bier et al. [5] considered that S and P are glucose and ATP, respectively. Their analysis was focused on temporal behaviour of two coupled glycolytic oscillators.

To describe the coupling, they employed the phenomenological terms proposed by Prigogine and Lefever [8], i.e.,

$$\frac{dx_1}{dt} = F - k_1 x_1 y_1, \quad (28)$$

$$\frac{dy_1}{dt} = 2k_1 x_1 y_1 - \frac{k_2 y_1}{K + y_1} + v(y_2 - y_1), \quad (29)$$

$$\frac{dx_2}{dt} = F - k_1 x_2 y_2, \quad (30)$$

$$\frac{dy_2}{dt} = 2k_1 x_2 y_2 - \frac{k_2 y_2}{K + y_2} + v(y_1 - y_2), \quad (31)$$

where x_1 , y_1 , x_2 and y_2 are the S and P concentrations in the first and second cell, respectively; F is the rate of the S supply into the cells; k_1 is the rate constant of step (26); and k_2 and K are the Michaelis-Menten constants for step (27).

In our treatment, steps (26) and (27) are considered to be ingredients of a generic model incorporating the P transport out of the cells. The corresponding kinetic equations are as follows [cf. Eqs. (22) and (24)]

$$\frac{dx_1}{dt} = F - k_1 x_1 y_1, \quad (32)$$

$$\frac{dy_1}{dt} = 2k_1 x_1 y_1 - \frac{k_2 y_1}{K + y_1} - k_d y_1 + v y_2, \quad (33)$$

$$\frac{dx_2}{dt} = F - k_1 x_2 y_2, \quad (34)$$

$$\frac{dy_2}{dt} = 2k_1 x_2 y_2 - \frac{k_2 y_2}{K + y_2} - k_d y_2 + v y_1, \quad (35)$$

where k_d and v are the rate constants defined by Eqs. (14) and (23) [the other designations are as in Eqs. (28–31)].

Comparing Eqs. (28–31) and (32–35), it is appropriate to notice that the coupling in the latter case has a slightly different structure from the former case, since it does not depend on the difference between y_1 and y_2 . This implies that the frequency of oscillations in the synchronized state is not the same when cells are completely separated. This effect is a consequence of the fact that in Eqs. (32–35) the metabolite diffusion from the cells results not only in the coupling, which disappears at $l \rightarrow \infty$, but also in the metabolite losses which remain finite at $l \rightarrow \infty$. In Eqs. (28–31), the latter effect is lacking. For physically reasonable values of the coupling coefficients, the corresponding difference in the frequency of oscillations is however minor.

Figure 2. Oscillatory kinetics in a single cell in the case when there is no P diffusion out of the cell [according to Eqs. (32) and (33) with $F = 5$, $k_1 = 0.2$, $k_2 = 50$, $K = 10$, $k_d = v = 0$ and the initial conditions corresponding to the limit cycle]. The thick and thin lines show the S and P concentrations (x and y), respectively.

Figure 3. As Figure 2 but in the case when P diffuses (with $k_d = 1$) out of the cell.

Figure 4. Oscillatory kinetics calculated for two communicating cells by using Eqs. (32–35) with $F = 5$, $k_1 = 0.2$, $k_2 = 50$, $K = 10$, $k_d = 1$, and $v = 0.1$. The thick and thin lines show the S and P concentrations (x and y), respectively. Initially, both cells are in the ‘out-of-phase’ regime near the limit cycle.

To integrate Eqs. (32–35), we have used a set of dimensionless rate constants, $F = 5$, $k_1 = 0.2$, $k_2 = 50$, $K = 10$, corresponding to limit-cycle oscillations.

First, we show (Figure 2) typical oscillations in a single cell with no P diffusion out of the cell. This case is described by Eqs. (32) and (33) with $k_d = v = 0$.

Secondly, we demonstrate (Figure 3) oscillatory kinetics in a single cell with P diffusion out of the cell. This case is described by Eqs. (32) and (33) with $k_d \neq 0$ and $v = 0$. In our calculations, we have used $k_d = 1$. This rate constant is relatively

Figure 5. As Figure 4 for $v = 0.05$.

small and accordingly the effect of P diffusion out of the cell on oscillations is not strong (cf. Figures 2 and 3).

To show oscillatory kinetics in two communicating cells (Figure 4), we have integrated Eqs. (32–35) with $k_d = 1$ and $v = 0.1$ (note that according to our analysis presented in the preceding section we should have $v \ll k_d$). Initially, both cells were chosen to be in the ‘out-of-phase’ regime near the limit cycle. Synchronization of oscillations is seen to be relatively slow. Mathematically, this is connected with a low value of v compared to the values of other rate constants. Physically, the reason of slow synchronization is that the perturbation of the P concentration near one cell due to the diffusion flux from another cell is weak.

With increasing distance between the cells, the coupling coefficient v decreases [Eq. (23)]. If for example the distance becomes two times longer, the coupling

Figure 6. Oscillatory kinetics obtained for two communicating cells by employing Eqs. (28–31) with $F = 5$, $k_1 = 0.2$, $k_2 = 50$, $K = 10$, and $v = 0.1$.

coefficients should be two times lower. To illustrate the corresponding changes in the reaction kinetics, we have reduced the coupling coefficient from $v = 0.1$ (Figure 4) down to $v = 0.05$ (Figure 5). The time characterizing synchronization becomes in the latter case about two times longer (cf. the lower panels of Figures 4 and 5).

Finally, we have compared the reaction kinetics predicted by Eqs. (32–35) (Figure 4) with those obtained by using Eqs. (28–31) (Figure 6) with the same values of the model parameters. Although the reaction kinetics are in these cases slightly different, the shape of the transition to the asymptotic synchronized regime is nearly the same (cf. the lower panels of Figures 4 and 6). This agreement justifies using the phenomenological equations for describing synchronization of oscillations.

3. Indirect Communication between Adsorbed Cells

In the Introduction, we reproduced the conventional system of equations describing two equivalent, two-variable ensembles of oscillators interacting via the bath. Such equations are applicable to two ensembles of dissolved cells provided that the reaction kinetics inside each ensemble are synchronized. In this section, we discuss how those equations should be modified if cells are adsorbed at the liquid-solid interface.

In our treatment, we consider that the cells are located at the interface at random. The interaction of the metabolite, diffusing out of the cells, with the interface is assumed to be negligible (no adsorption). This means that the metabolite, leaving the cells, diffuses primarily away from the interface. The cell-cell communication is considered to be indirect, i.e., to occur via the metabolite concentration, $n(z, t)$ (z is the coordinate perpendicular to the interface), formed due to metabolite diffusion from the cells. In this case, the rate of metabolite diffusion from a single cell can be calculated by using the approach similar to that described in the beginning of Sec. 2.2. In particular, the total diffusion flux from a single cell and the flux per unit volume of the cell are given by [cf. Eq. (13)]

$$J_1(t) = k_d V [n_1(t) - n(0, t)], \quad (36)$$

and

$$j_1(t) = J_1(t)/V = k_d [n_1(t) - n(0, t)], \quad (37)$$

where k_d is the rate constant defined by Eq. (14), $n_1(t)$ the metabolite concentration inside the cell, and $n(0, t)$ the metabolite concentration outside the cells near the interface ($z = 0$ corresponds to the interface). Thus, the equations for the concentrations of the diffusing metabolite inside the cells, belonging to the two ensembles, can be represented as [cf. Eq. (15)]

$$dn_1(t)/dt = g(n_1(t)) + k_d [n(0, t) - n_1(t)], \quad (38)$$

$$dn_2(t)/dt = g(n_2(t)) + k_d [n(0, t) - n_2(t)], \quad (39)$$

where $n_1(t)$ and $n_2(t)$ are the metabolite concentrations inside the cells belonging to ensemble 1 and 2, respectively, and $g(n_1)$ is the function defining the metabolite internal dynamics (as already noted in Sec. 2.2, this function actually depends not only on n_1 but also on the other metabolite concentrations).

To calculate $n(0, t)$, we should integrate the diffusion equation

$$\frac{\partial n(z, t)}{\partial t} = D \frac{\partial^2 n(z, t)}{\partial z^2} - \gamma n(z, t), \quad (40)$$

where γ is the rate constant of the metabolite removal due to reaction(s) outside the cells. The boundary condition for this equation is as follows

$$-D \left. \frac{\partial n(z, t)}{\partial z} \right|_{z=0} = J(t), \quad (41)$$

where [cf. Eq. (36)]

$$J(t) = N[J_1(t) + J_2(t)] \equiv k_d V N[n_1(t) + n_2(t) - 2n(0, t)] \quad (42)$$

is the total metabolite diffusion flux out of cells per unit interface area (N is the surface concentration of cells in each ensemble).

A formal solution to Eq. (40) with the boundary condition (41) is given by

$$n(z, t) = 2 \int_{-\infty}^t J(t') G(z, t - t') dt', \quad (43)$$

where

$$G(z, \tau) = (4\pi D\tau)^{-1/2} \exp\left(-\frac{z^2}{4D\tau} - \gamma\tau\right) \quad (44)$$

is the Green function. Substituting expressions (42) and (44) into Eq. (43) yields

$$n(0, t) = \frac{k_d V N}{(\pi D)^{1/2}} \int_{-\infty}^t [n_1(t') + n_2(t') - 2n(0, t')] \frac{\exp[-\gamma(t - t')]}{(t - t')^{1/2}} dt'. \quad (45)$$

Eqs. (38) and (39) combined with Eq. (45) make it possible to describe synchronization of oscillations of two ensembles of adsorbed cells (similar equations can be used to treat bath-mediated interaction of several ensembles). This case is seen to be more complex than the corresponding case of synchronization of oscillations in the bulk of solution (see the Introduction), because Eq. (45) taking into account indirect communication between adsorbed cells is integral.

Explicit numerical integration of Eqs. (38), (39) and (45) is beyond our present goals. To illustrate application of these equations, we confine our analysis by treatment of the asymptotic behaviour of the system when the oscillations are already synchronized, i.e., $n_1(t) = n_2(t)$. Specifically, we are interested in the relationship between the average (over the oscillation period) metabolite concentrations, $\langle n_1(t) \rangle$ and $\langle n(0, t) \rangle$, inside and outside the cells. To obtain this relationship, we can replace $n_1(t)$, $n_2(t)$ and $n(0, t)$ in Eq. (45) by the average values of these variables. This procedure yields

$$\langle n(0, t) \rangle = 2k_d V N \langle n_1(t) \rangle / [(\gamma D)^{1/2} + 2k_d V N]. \quad (46)$$

According to the latter equation, the concentrations $\langle n_1(t) \rangle$ and $\langle n(0, t) \rangle$ are nearly equal if $(\gamma D)^{1/2} \ll 2k_d V N$. Otherwise, $\langle n_1(t) \rangle$ is appreciably higher than $\langle n(0, t) \rangle$.

4. Conclusion

We have analyzed in detail direct diffusion-mediated communication between two cells and also derived a general integral equation describing indirect communication between adsorbed cells. The results obtained may be useful for the future experimental studies of kinetic oscillations in the metabolism of adsorbed cells.

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