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Spatiotemporal dynamics of glycolytic waves provides new insights into the interactions between immobilized yeast cells and gels ☆

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ABSTRACT

The immobilization of cells or enzymes is a promising tool for the development of biosensors, yet the interactions between the fixative materials and the cells are not fully understood, especially with respect to their impact on both cell metabolism and cell-to-cell signaling. We show that the spatiotemporal dynamics of waves of metabolic synchronization of yeast cells provides a new criterion to distinguish the effect of different gels on the cellular metabolism, which otherwise could not be detected. Cells from the yeast *Saccharomyces carlsbergensis* were immobilized into agarose gel, silica gel (TMOS), or a mixture of TMOS and alginate. We compared these immobilized cells with respect to their ability to generate temporal, intracellular oscillations in glycolysis as well as propagating, extracellular synchronization waves. While the temporal dynamics, as measured by the period and the number of oscillatory cycles, was similar for all three immobilized cell populations, significant differences have been observed with respect to the shape of the waves, wave propagation direction and velocity in the three gel matrices used.

1. Introduction

The immobilization of microorganisms has attracted increased scientific interest, since it provides a promising tool for biotechnological applications [1–4]. In addition, immobilized living cells may be used for the construction of biosensors, e.g. in the food industry or in the monitoring of environmental pollution. Several immobilization procedures have been reported [5–12], each having its advantages and drawbacks. One problem concerns the dynamics and regulation of the metabolism, which seems to be affected in the immobilized microorganisms [11–14]. A fast and easy method to test for such perturbations is related to glycolytic oscillations. They are easy to detect by measuring the NAD(P)H fluorescence of the immobilized cells and information about the metabolic state of the cells can be derived from characteristic parameters as for example the period and amplitude of the oscillations [15,16]. Monitoring of fluorescence intensities of NAD(P)H during aerobic–anaerobic transition has also been used to determine the viability of yeast cells immobilized in transparent matrices. Immobilized cells from the exponential and the stationary growth phases were monitored [13].

Macroscopic glycolytic oscillations of yeast cell populations result from metabolic synchronization of the individual cells [16–18]. These oscillations have a short period of about 0.5–1 min and occur when starved cells of *Saccharomyces cerevisiae* are supplemented with glucose pulses, either after switching to anaerobiosis [19] or after addition of cyanide, i.e. at energy limiting conditions. Acetaldehyde has been found to act as the signaling compound for this type of cell–cell synchronization. Sustained glycolytic oscillations occur only when the yeast cells are harvested during the diauxic shift [20].

These experiments have been performed with stirred cell suspensions. However, oscillatory systems may generate spatiotemporal patterns when the oscillatory reaction is coupled to mass transport processes, such as diffusion [21,22]. As this is the case in gel-fixed yeast cell layers, the glycolysis in entrapped yeast cells may give rise to a series of waves and patterns, which are well known from self-organized reaction–diffusion systems [22–25]. Metabolic synchronization of gel-fixed cell populations may thus occur via traveling waves of the signaling compound, which cannot form in stirred cell suspensions. Results from chemical systems demonstrate that the propagation dynamics as well as the shape of such traveling reaction–diffusion waves contains information about the state of the system [26,27]. Hence, these waves may be of relevance for biological information processing [28,29], and as such also for biotechnological applications.

The first experimental evidence for the generation of reaction–diffusion waves in glycolysis came from experiments with yeast extract [30,31]. There, traveling NADH and proton waves could be observed during oscillatory glycolysis. In these experiments, the necessary coupling between reaction and diffusion was achieved by

☆ We dedicate this article to Dr. Thomas Mair, who sadly passed away during the publication process of this article.

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performing the glycolytic reaction in a gel matrix. A drawback of these investigations is that they have been conducted under batch conditions. Since the generation of oscillations and reaction–diffusion waves requires nonequilibrium conditions, such experiments are best run in open reactors [32].

Recently, Bagyan et al. monitored reaction–diffusion waves in the yeast extract immobilized in an agarose gel mounted into an open spatial reactor [33]. They could show that in the case of the yeast extract local desynchronization plays an important role for the wave dynamics [34]. Lavrova et al. have simulated glycolytic wave propagation in this open spatial reactor in order to explain some of the phenomena observed at low protein concentrations [35]. Due to the possibility of maintaining the reaction under stationary nonequilibrium conditions, this reactor should also be useful for the measurement of oscillations and waves in yeast cell suspensions.

In the present work, we show first results about the application of this type of reactor for cell suspensions immobilized in gels with a focus of the impact of various gel matrices on the dynamics of glycolytic patterns. We aimed at investigating (i) the effect of the immobilizing gel medium on the generation of glycolytic oscillations in yeast cells and (ii) the spatiotemporal patterns that may arise in immobilized cell layers, i.e. when there is a coupling between reaction and diffusion. Three different gel matrices were used: agarose, pre-polymerized tetramethoxysilane (TMOS) and compositions of pre-polymerized tetramethoxysilane with alginate (alginate/TMOS).

2. Materials and methods

2.1. Growth of yeast

The yeast *Saccharomyces carlsbergensis* ATCC 9080 was cultivated aerobically in a rotary shaker at 180 rpm in liquid semi-synthetic minimal media [36] at 28 °C. After complete consumption of glucose the cells were harvested by centrifugation at 5000 ×*g* at 21 °C for 5 min and were washed twice with distilled water. The cells were resuspended in potassium phosphate buffer (0.1 M KH_2PO_4, pH 6.5) yielding cell suspensions in concentrations of 10, 20 or 30% (wet weight per volume). Subsequently, these cell suspensions were starved at 21 °C for at least 6 h until further use.

2.2. Gel preparation and immobilization procedure

After at least 6 h of starvation, the yeast cells were immobilized in one of the following three gel matrices that were prepared as follows.

2.2.1. 1% agarose gel

Agarose (Type VII; Sigma-Aldrich) (0.03 g) in potassium phosphate buffer (1.5 mL of 0.1 M KH_2PO_4, pH 6.5) was heated for 5 min at 70 °C. The solution was placed in a water bath at 28 °C. After 2 min, the cell suspension (1.5 mL; incubation at 28 °C) was carefully mixed with agarose and subsequently this mixture was placed between two glass plates (20 cm × 15 cm) separated by 1.5 mm thick spacers. The gelation process took place during 15 min at ca. 0 °C. A flat surface of the gel was obtained by placing a 5 kg weight on the upper glass plate during the gelation process. Thereafter a circular piece (diameter 24 mm) of the gel slide was cut out and placed on a black nitrocellulose membrane (Millipore) of 0.8 μm pore size [33].

2.2.2. Silica gel

Tetramethoxysilane (TMOS; Fluka) was mixed with distilled water and HCl (0.1 M) in TMOS:H_2O:HCl molar ratio = $1:5:10^{-2}$, to form a clear solution. This solution was left to pre-polymerize for 24 h at 4 °C [13,37]. The pre-polymerized TMOS was used for immobilization in the following way: the cell suspension (1 mL) was mixed with 0.05 M NaOH (0.5 mL) and with pre-polymerized TMOS (0.5 mL). This mixture was vortexed and poured on the black nitrocellulose membrane which was covered with an appropriate spacer ring (diameter 24 mm, Teflon). After gelation (within ca. 1–2 min, at room temperature), planar film disks (of 24 mm diameter and 15 mm thickness) of immobilized cells were very quickly cut out and the spacer ring was removed.

2.2.3. Alginate/TMOS matrix

At first a stock solution of sterile 3.2% (w/v) sodium alginate (Sigma-Aldrich, type IV, practical grade) was prepared as follows: the alginate and distilled water were sterilized separately (20 min, 120 °C, 0.1 kPa). Thereafter, the sterilized alginate was dissolved in the sterilized distilled water under sterile conditions and stored at 4 °C until further use. For preparation of the gel the cell suspension (1 mL) was mixed with 0.05 M NaOH (0.5 mL), dissolved alginate (0.167 mL) and pre-polymerized TMOS (0.333 mL). Then this mixture was very quickly vortexed and poured on the black nitrocellulose membrane covered with an appropriate spacer ring (diameter 24 mm, Teflon). After gelation (within ca. 1–2 min at room temperature) the biofilm was immersed into a $CaCl_2$ solution (7 g/L) for 30 min [13,37]. Subsequently, planar (disk) films of immobilized cells (24 mm diameter and 15 mm thickness) were cut out and the spacer ring was removed.

2.2.4. Monitoring of glycolytic activity

The glycolytic activity of yeast cells was measured under two different conditions: (i) in stirred suspensions in a conventional spectrophotometer and (ii) in gel-fixed suspensions in an open spatial reactor, i.e. at unstirred conditions. For both systems the glycolytic activity was measured by the NAD(P)H fluorescence at excitation and emission wave lengths λ = 366 and 460 nm, respectively.

(i) Glycolytic oscillations in stirred cell suspensions: 1.9 mL of 10% (w/v) yeast cells was placed in a spectrofluorimeter (AB2 Luminescence spectrophotometer, SLM AMINCO, Milton Roy Co., USA). The cells were stirred in a thermostatically regulated cuvette at 21 °C (Hellma, Germany). Glycolytic oscillations were initiated by addition of glucose (0.1 mL; 1 M) into the cell suspension.

(ii) Glycolytic oscillations and waves from immobilized cells: the immobilized yeast cell disks were placed in an open spatial reactor (Fig. 1). The open spatial reactor consists of diffusive gel layer in contact with the content of a continuous-flow stirred tank reactor (CSTR). This CSTR acts as a feeding reservoir for the immobilized cell layer. The immobilized yeast cells were separated from the CSTR by an Anodisc membrane, with 20 nm pore size (Whatman). The black nitrocellulose membrane and the immobilized yeast cell disk were placed on top of this Anodisc membrane. Glycolytic oscillations of the immobilized yeast cells were initiated by feeding a solution containing 60 mM glucose, 0.1 M KH_2PO_4, pH 6.5, and 0.1 mM acetaldehyde into the open spatial reactor. Spatiotemporal concentration changes of NAD(P)H (i.e. NAD(P)H waves) were monitored by means of an image intensified CCD camera (Corail, Optronics) and the resulting movies digitized at a temporal resolution of 2 images per second with a frame grabber card using a standard personal computer. For further details on the experimental setup, see Bagyan et al. [33].

For analysis of the local temporal dynamics, the intensity of the NAD(P)H fluorescence (gray levels) of a selected area (20 × 20 pixels) from each image of the movie was averaged and plotted as a function of time. For analysis of the spatiotemporal dynamics of the glycolytic waves a time–space plot of propagating NAD(P)H waves was constructed from the recorded movies along the selected lines. The intensity along the selected line from each image of the movie was

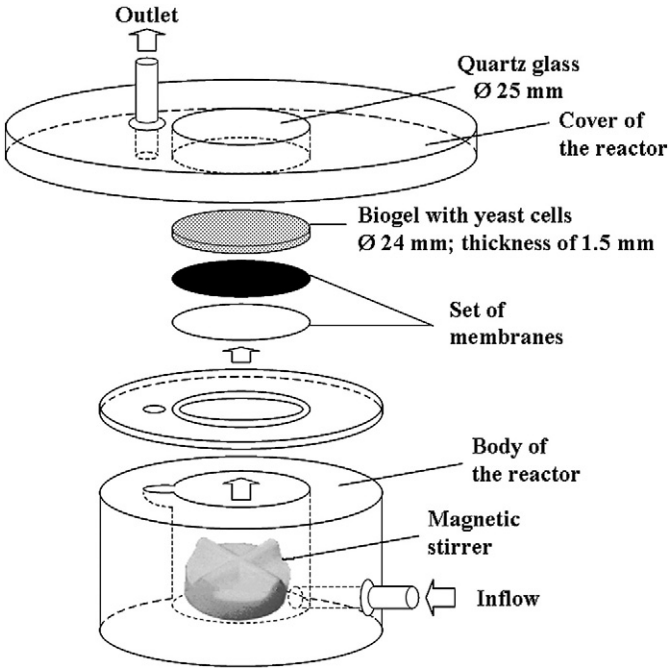

Fig. 1. Sketch of the open spatial reactor used to perform the experiments with gel-immobilized yeast cell layers.

stacked on top of each other, resulting in the time–space plot, which displays the spatiotemporal dynamics of propagating waves along this line. The reciprocal of this line corresponds to the wave velocity.

3. Results

3.1. Induction of glycolytic oscillations in stirred yeast cell suspensions

Addition of glucose to starved yeast cells induces glycolytic oscillations, provided that the cells are under energy limiting conditions. At the chosen growth conditions of the yeast, a starvation period of about 3–5 h is required, when stirred yeast cells are investigated (Fig. 2). During the starvation period the amplitude of the oscillations increases from 0.12 to 0.31 a.u. until it reaches a plateau after about 6–7 h (Fig. 3). On the other hand, the oscillatory period remains relatively constant at 30 ± 4 s. These values serve as a reference for the experiments with the immobilized cells. For all

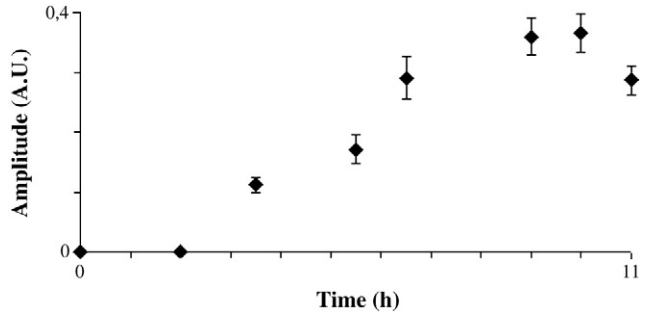

Fig. 3. Increase of the amplitude of glycolytic oscillations in stirred suspensions of *S. carlsbergensis* (cell concentration: 10% wet (w/v)) as a function of the starvation time. Oscillations have been measured by NAD(P)H fluorescence. The mean value is calculated from 10–12 amplitudes. Error bars show ± standard deviation.

immobilization experiments we used yeast cells that had been starved for 9 h.

3.2. Glycolytic oscillations of immobilized yeast cells in gel matrices

Gel-fixed yeast cells represent a diffusive layer and thus open the possibility to investigate their ability to generate spatiotemporal patterns of reaction–diffusion type in an open system. To this purpose, the gel layer of the fixed cells is coupled via a membrane to a continuous stirred tank reactor (CSTR), which acts as a feeding reservoir [33] (Fig. 1). The generation of glycolytic patterns in yeast cell layers is expected to occur via extracellular acetaldehyde waves [38]. These extracellular waves, however, can be visualized only indirectly, e.g. via intracellular NAD(P)H. Changes in the intracellular NAD(P)H fluorescence can be seen as an indicator for the concentration changes in the extracellular acetaldehyde because the oscillations of these two compounds exhibit a fixed phase relation [18]. Thus, we have taken the spatiotemporal dynamics of intracellular NAD(P)H fluorescence as an indirect measure of extracellular acetaldehyde waves.

Concomitant application of glucose and acetaldehyde to the gel-fixed yeast cells induces glycolytic oscillations in these cells. Acetaldehyde has been applied together with glucose in order to avoid a drop of its concentration below a critical value [18]. The analysis of local fluorescence changes reveals the generation of glycolytic oscillations, irrespective of the type of gel used (Fig. 4). The period of the glycolytic oscillations (53 s) as well as the number of oscillatory cycles (13–15) is nearly the same in the three gels studied (Fig. 5).

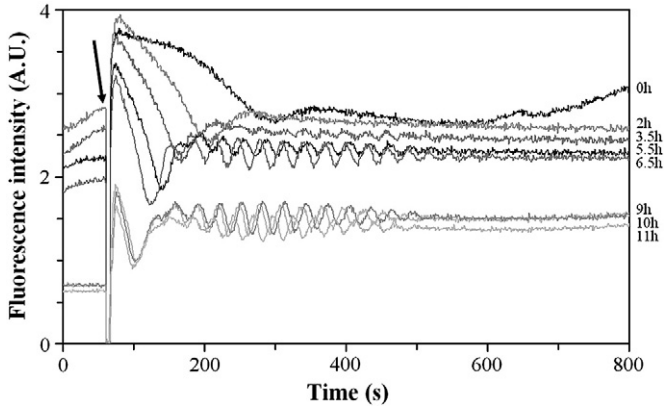

Fig. 2. Glycolytic oscillations in stirred suspensions of *S. carlsbergensis* (cell concentration: 10% wet (w/v)) after different times of starvation. Glycolysis was measured by NAD(P)H fluorescence in a conventional spectrofluorimeter. The oscillations were initiated by addition of glucose to the cell suspension (marked by arrow). The yeast cells had been starved for 0–11 h.

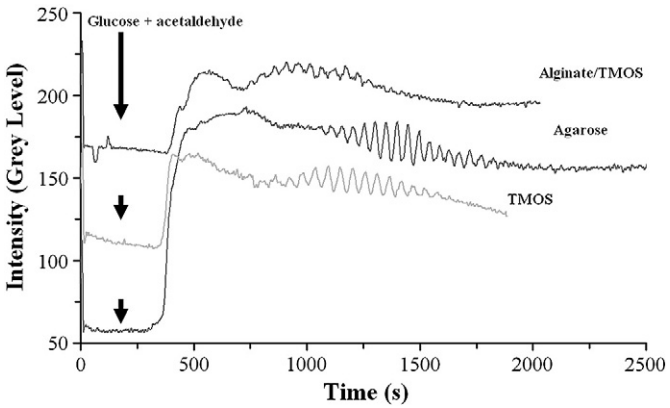

Fig. 4. Glycolytic oscillations of immobilized cells of *S. carlsbergensis* (10% w/v) in gel matrices. The glycolytic oscillations of the gels were measured in the open spatial reactor (stirring rate: 500 rpm, feeding rate: 74 mL/h and temperature 21 °C). The cells were immobilized after 9 h of starvation and the oscillations were initiated by addition of glucose and acetaldehyde (at time 180 s, marked by the arrow).

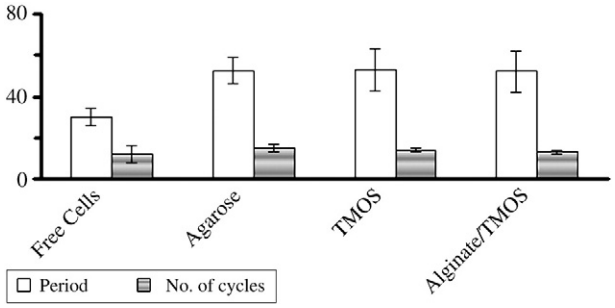

Fig. 5. Comparison of the kinetic values of the glycolytic oscillations from the cells shown in Fig. 2 (after 9 h starvation) and Fig. 4. Period is given in seconds.

However, a comparison of gel-fixed cells with stirred cell suspensions demonstrates a 50% drop in the period for the stirred cells, whereas the number of cycles remains nearly unaffected (13–15 vs. 12). Since the period is also related to temporal events we may take this change as an indicator for the slower diffusive processes in the gels, which are overcome by stirring in experiments with cell suspensions.

3.3. Spatiotemporal dynamics

In the extended gel-immobilized cell system, we also followed the spatiotemporal distribution of NAD(P)H. The signals are very weak so that the signal to noise ratio of the images is quite poor. However, the evaluation of the movies after image processing (background subtraction, contrast enhancement) revealed the spontaneous formation of propagating patterns. They can be seen as white stripes or spots (increased NAD(P)H concentration) on a dark background. To improve visualization of the waves we have marked them by dashed lines (Fig. 6A). The direction of wave propagation is indicated by an arrow. With agarose as fixative (Fig. 6A and B) we observe multiple wave sources which periodically emit waves that eventually combine to one large wave. Similar wave patterns are observed when the yeast cells were immobilized in TMOS (Fig. 6C and D) or in alginate/TMOS. No significant differences in the wave velocity were found in agarose and TMOS (both at 130 ± 20 μm/s) gels, whereas in alginate/TMOS the propagation velocity is about twice as large (240 ± 10 μm/s).

A further difference between yeast cells immobilized in silica (TMOS) and agarose gel at the one side and cells entrapped in alginate/TMOS gel is the direction of propagation of the glycolytic waves (Fig. 6). While waves in agarose and silica gels were found to propagate from the center of the reactor outwards, waves of cells in alginate/TMOS gels propagate in the opposite direction, i.e. inwardly.

The time–space plots (Fig. 7) constructed from the movies at three different spatial locations (marked as white lines in Fig. 6B) demonstrate the impact of the type of gel on the wave propagation dynamics. On these time–space plots horizontal lines are due to oscillations in the medium, while inclined white lines originate from propagating NAD(P)H waves. Whereas in agarose the spatial patterns

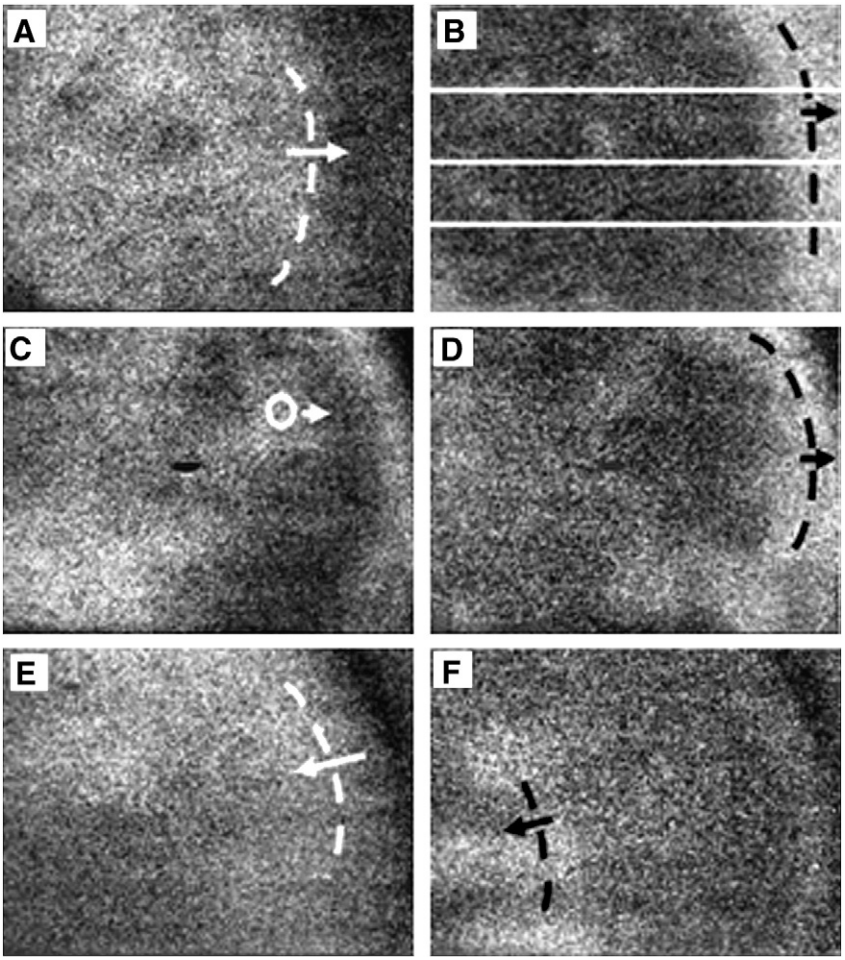

Fig. 6. Propagating waves of glycolytic activity in immobilized yeast cell layers after glucose addition, as measured by intracellular NAD(P)H fluorescence. Immobilization was performed in agarose (A, B), TMOS (C, D) and alginate/TMOS (E, D). For improved visualization the wave fronts are marked by dashed lines (in C with a circle) and the propagation direction by an arrow. In B the three different locations (1/3, 1/2, and 2/3 from top to bottom) at which time–space plots have been constructed are marked by horizontal full lines. The time interval is 88 s for A to B, 78 s for C to D and 86 s for E to F. The *x*-axis of each image corresponds to 12 mm.

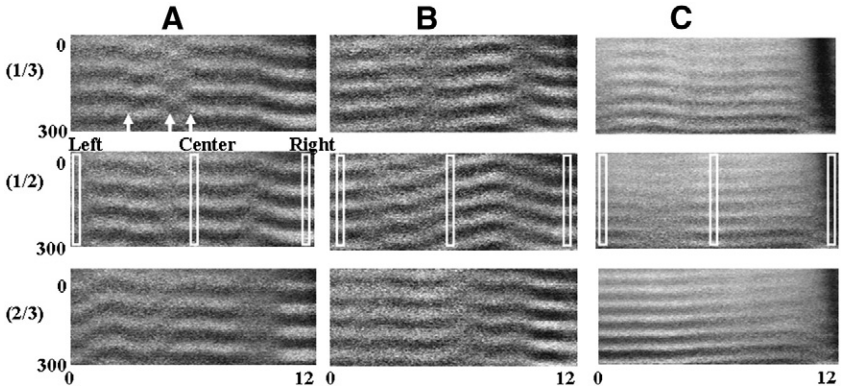

Fig. 7. Time–space plots of propagating waves in immobilized yeast cell layers (cell concentration: 10% (w/v)) in (A) agarose, (B) silica (TMOS) gel and (C) alginate/TMOS gels. The different locations at which the time–space plots were taken (1/3, 1/2 and 2/3) are shown in Fig. 6B. The white arrows in A indicate wave interactions due to annihilation and wave break up. The rectangles show the positions at which the time traces, shown in Fig. 8, have been taken.

are quite complex even showing several wave dislocations (see arrows, Fig. 7A), the patterns in the alginate/TMOS gel are very regular with only minor spatial differences (Fig. 7C). In TMOS alone we can see regular propagating waves mainly in the middle of the gel (Fig. 7B, 1/2), whereas at the borders the waves propagate at lower velocity.

The impact of the gel composition on the propagation velocity of the waves can be also deduced from the time traces taken from the time–space plots of Fig. 7, which are plotted in Fig. 8. On average we find a phase shift between the center and the border of the gel of 7.5 s for agarose (Fig. 8A), 25.9 s for TMOS (Fig. 8B) and 0.5 s for alginate/TMOS (Fig. 8C).

4. Discussion

The results presented here demonstrate for the first time the generation of propagating synchronization waves in yeast cell immobilized into layers of gels. The results also show that the open spatial reactor, used to perform these experiments, is well suited for the investigation of spatiotemporal pattern formation, not only in yeast extract [33–35] but also in yeast cell layers.

Although the use of the open reactor is intended at maintaining the cell population in a sustained oscillatory state, we find only transient oscillations (Fig. 4). This indicates that the cells do not settle on an oscillatory state because some drifts in the reaction conditions cannot be fully controlled. Possibly, either substances required to maintain the glycolytic NAD(P)H oscillations leak out of the gel layer, or substances that inhibit the oscillations accumulate in the gel. Acetaldehyde itself may be a potential candidate for both of these possibilities since a drop below or an increase above the oscillatory concentration window abolishes the oscillations [18].

Supply of glucose to the immobilized cells induces not only temporal glycolytic oscillations but also traveling waves (Fig. 6). This means that there is a coupling between the intracellular oscillatory production of acetaldehyde, which is released into the extracellular medium, and the extracellular transport of the messenger acetaldehyde by diffusion. The propagation dynamics of the traveling waves consequently depend on both, the oscillatory reaction and the transport step. Thus, possible interactions of the gels with the metabolism of the cells (oscillatory glycolysis) as well as with the extracellular signaling compounds (extracellular diffusion of acetaldehyde) may be encoded in these waves.

When comparing the characteristics of the temporal glycolytic NAD(P)H oscillations observed in gel-immobilized yeast cells to those of cells in suspension, we observe that the period of oscillations is twice as large in the gel-immobilized cells than in the suspended ones (Fig. 5). Since the glycolytic oscillations are the result of an acetaldehyde-mediated synchronization between the cells [18], stirring of the cell suspensions provides an immediate distribution of this compound into the entire reactor, whereas in the gel the transport of this messenger is provided exclusively by molecular diffusion, which is much slower. Thus, the increased oscillatory period in the gels is caused by the slower diffusion of extracellular acetaldehyde. Similar results have been obtained by Doran and Bailey with cells from *S. cerevisiae*, suspended in phosphate buffer or immobilized in gelatin [11]. Although the immobilization procedure and the type of gel differed markedly from ours, a pronounced increase of the period of glucose induced oscillations in intracellular NAD(P)H for the cells immobilized in gelatin was also found. When measured at 25 °C, the period rose from 54 s for suspended cells to about 240 s for the immobilized cells [11].

In the following, we discuss the influence of the type of gel on both, the temporal and spatiotemporal dynamics of entrapped yeast cells. To this purpose the (i) periods of the temporal oscillations as well as (ii) the direction and (iii) speed of the propagating waves will be discussed. Interestingly the temporal dynamics show no difference among the different gels, although the three gels studied in the present article have different pore sizes. Obviously, the differences in extracellular diffusion in the gel types studied are not sufficiently large to significantly alter the period of oscillations.

The picture changes, when it comes to comparing the spatiotemporal wave patterns of NAD(P)H concentration in the three gels studied (agarose, TMOS, and alginate/TMOS). The wave patterns are relatively similar to each other, thus demonstrating the robustness of oscillatory glycolysis arising even from yeast cells immobilized in gels. Nevertheless, significant differences were found when it comes to the wave propagation direction and velocity, which depend sensitively on the identity of the immobilizing gel. In fact, glycolytic waves from cells entrapped in silica (TMOS) and agarose gels always propagated outwards, while the propagation direction in alginate/TMOS gels was always inward. The difference in wave propagation direction correlates with the behavior of the wave speed. Again, the wave speeds are similar in agarose and silica gel, while it is twice as high in alginate/TMOS. These observations suggest that the availability of acetaldehyde for the cells entrapped in agarose or silica gels on the one hand and in alginate/TMOS at the other hand differs. This may be due either to subtle differences in the transport efficiency of acetaldehyde, since such changes in the transport process have recently been shown to affect the direction of wave propagation [34,35], or to a different way of entrapping the cells in the gel matrices.

To better understand how the yeast cells are entrapped into the gel matrices, we have performed a preliminary screening electron microscopic (SEM) study, which indicates that the yeast cells are monodispersely entrapped in both agarose and silica gels. This contrasts with the alginate/TMOS gel, where the cells are found to form small clusters comprising a small number of cells. The formation

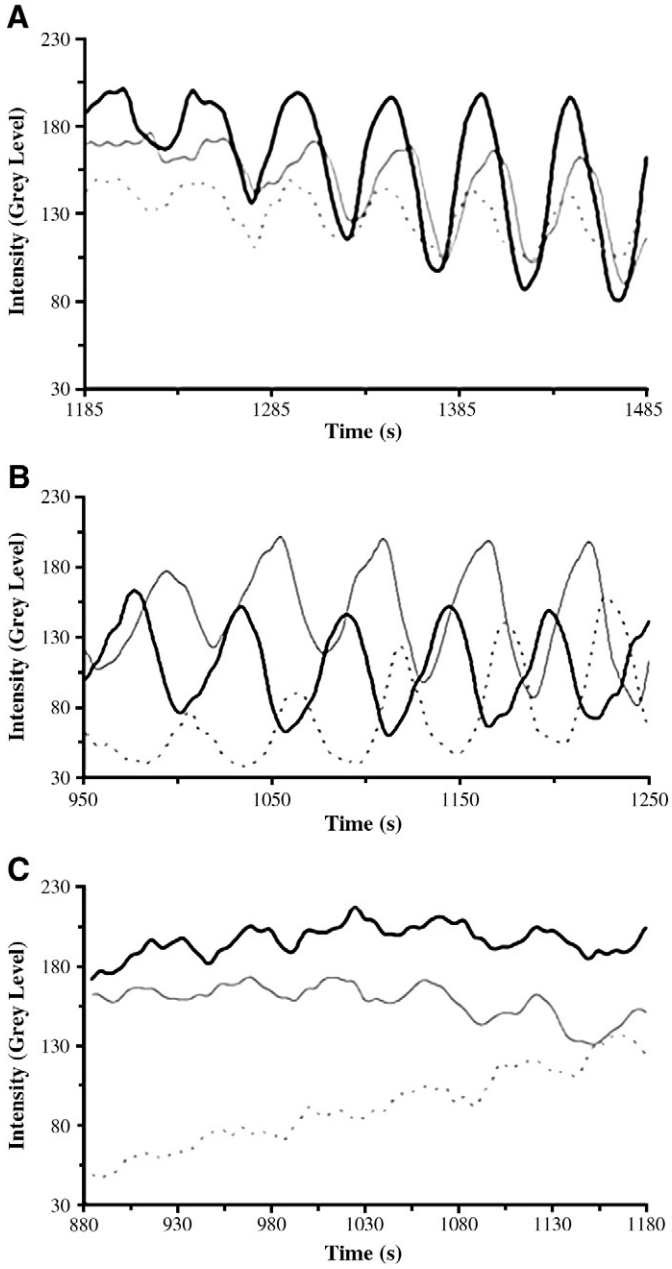

Fig. 8. Temporal dynamics of the local oscillations as taken from the time–space plots in Fig. 7 in (A) agarose, (B) silica (TMOS) and (C) alginate/TMOS gels. The variable locations at which these time traces have been obtained are marked by solid black lines (center), solid gray lines (left border) and dashed lines (right border).

of cell clusters in alginate/TMOS matrices may explain the different direction of glycolytic wave propagation in this matrix: in small clusters, the cells are packed tightly together, so that the acetaldehyde released from one cell immediately triggers the release of acetaldehyde of the other cells in the cluster. The clusters are randomly distributed in the gel, and thus act as randomly distributed nucleation sites for wave initiation. Unevenly, but randomly distributed clusters consequently favor the generation of waves that propagate inwardly.

The wave propagation speed is similar for cells entrapped in agarose and silica gels, while the wave velocity is significantly higher in alginate/TMOS gel. This is somewhat surprising, since this composite gel contains weak chemical bonds between the hydroxyl groups of the polymerized aqueous silica and the polysaccharide chains of alginate [39,40]. Therefore the alginate/TMOS gel would have been expected to have a considerably smaller pore size than TMOS alone or the agarose gel. In turn, this would than have led to a decreased diffusion and to a lower wave propagation velocity.

However, the more effective transport in alginate/TMOS gels seems to be linked to the cluster formation of yeast cells, which may locally lead to the release of a higher concentration of acetaldehyde than it is the case of monodispersely distributed cells (as in agarose or silica gels). Since the excitation of a yeast cell requires a superthreshold concentration of the messenger acetaldehyde, this threshold is reached sooner when higher concentrations of the messenger are present. This leads to a higher effective wave propagation velocity. The more homogeneous appearance of the wave propagation in the alginate/TMOS gel (Fig. 7) is a further indication for a more effective transport of acetaldehyde between the entrapped cells. These findings suggest that there is a stronger mutual coupling of the cells in the alginate/TMOS gel than in the other two gels. Additional support for a more effective acetaldehyde transport in the alginate/TMOS gel is provided by the phase shift between the center and the borders of the gel (Fig. 8). In alginate/TMOS there is nearly no phase shift (0.5 s), whereas in agarose we find a phase shift of 7.5 s and in TMOS of 26 s.

Bagyan et al. [33] found also phase shifts for glycolytic waves, however, in immobilized yeast extract, measured with the same open spatial reactor as the one used in the present study. Later, Lavrova et al. simulated these results and attributed the phase shifts to a slight inhomogeneous substrate inflow [35]. An inhomogeneous distribution of the cells, however, might have a similar effect as an inhomogeneous substrate inflow. Spatial desynchronization phenomena might be taken into consideration as well [34]. Fig. 8 also reveals a strong decrease of the amplitude in cells immobilized in alginate/TMOS. However, fluorescence is a relative measure and thus should be taken with care when different experimental conditions are involved.

In conclusion, our results demonstrate that spatiotemporal glycolytic oscillations can be generated in yeast cells that are immobilized in gel layers. This is in good agreement with recent studies where such waves were also seen in sedimented (but not fixated) cells layers, and also with studies where gel-immobilized cell extracts were used. The spatiotemporal patterns of metabolic synchronization in gel-immobilized yeast cell layers provide a promising tool to investigate the interaction between the cells and the surrounding gel. Especially the wave velocity, direction, and the wave patterns seem to be suitable parameters for this purpose, since these characteristic features were found to depend sensitively from both the identity of and the physical parameters describing the embedding, fixating gel.

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