Metabolic synchronization of encapsulated yeast cells: Effect of gel matrices.

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INTRODUCTION

The understanding of the cell physiology of immobilized cells is relevant for increasing the productivity of biotechnological processes taking advantage of immobilized living cells. Immobilized cells have been used in a wide spectrum of applications, such as degradation of pollutants, production of ethanol, xylitol, etc., and as biosensors.

Whole-cell biosensors have been used for screening tests and monitoring of environmental pollution. For the use in biosensors, cells have been immobilized in matrices such as polyvinyl alcohol (Preininger C. et al., 1994), removable membranes (Naessens M. et al., 2000), or silica (Trogl J. et al., 2005; Gavlasova P. et al., 2008). The whole-cell biosensors are based on measurement of oxygen consumption (Preininger C. et al., 1994), concentration changes of substrates or products, production of bioluminescence (Trogl J. et al., 2005) and fluorescence. Yeast glycolytic oscillations might represent a novel approach in optical biosensors of chemical and physiological stressors. The dynamics of glycolysis, e.g. the frequency or the amplitude of glycolytic oscillations can provide information about changing environmental conditions and may thus lead to novel types of biosensors.

Glycolysis is the first pathway in a cell for the degradation of hexoses. Its role is to provide energy and precursors for other metabolic pathways. Under energy limiting conditions the glycolytic pathway exhibits oscillatory dynamics. Sustained oscillations require an active synchronization between the cells. Acetaldehyde has been identified as the main signaling compound for such active synchronization mechanism in yeast cells, which operates under certain conditions (Richard P., 2003; Ghosh A. et al., 1964). Glycolytic oscillations in yeast cells are an item of longstanding research (Ghosh A. et al., 1964; Betz A., 1966; Hess B. et al., 1968; Danø S. et al. (1999).

The glycolytic pathway exhibits oscillatory behavior under anaerobic conditions with damped period between 30 – 90 s. Yeast cells grown aerobically can exhibit oscillations with a frequency of 1 – 40 h (Richard P. (2003). Although glycolytic oscillations of yeast cells have been studied thoroughly, oscillations of immobilized cells have been described only in a few papers (Doran M.P. et al., 1987; Poulsen A.K. et al. (2007).

In this work we tested agarose, silica and silica-alginate gels for immobilization of yeast cells with respect to their capability to support oscillations and waves of glycolysis.

MATERIAL AND METHODS

Microorganism and resuspension procedure

The yeast *Saccharomyces carlsbergensis* ATCC 9080 obtained from the University of Magdeburg (Germany), was cultivated aerobically in liquid semi-synthetic minimal media in two steps (Hess B. et al. (1968). The temperature for growth was 28°C and the speed of the rotary shaker was 180 rpm. The cells were harvested after total consumption of glucose and centrifuged at 21°C, at 5000 rpm, for 5 minutes and were washed twice in distillated water. The cells were resuspended in potassium phosphate buffer (0.1 mol L^{-1}, pH 6.5) yielding cell suspensions in concentrations of 10, 20 and 30% wet cell mass weight per volume.

Glycolytic oscillations in cell suspension

The glycolytic oscillations of yeast cells in cell suspensions, were measured (at every hour for 11 hours) as the NAD(P)H fluorescence (A.U.) at excitation and emission wavelengths λ= 366 and 460 nm, respectively (AB2 Luminescence spectrophotometer, SLM AMINCO, Milton Roy Company, USA), in a stirred and thermostatically regulated cuvette (Hellma, Germany). The glycolytic oscillations (at 21°C, aerobic condition) were initiated by addition of glucose solution (0.1 mL; 1 mol L^{-1}) into cell suspension (1.9 mL), at time t = 60 s after start of measurement.

Gel preparation and immobilization procedure

After a minimum of 6h of starvation, the yeast cells in cell suspensions were immobilized in three gel matrices, prepared as follows:

1% agarose gel: **Agarose** (Type VII; Sigma-Aldrich, CAS 9012-36-6) (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 the agarose and subsequently this mixture was placed between 2 glass plates (20 cm x 15 cm) separated by 1.5 mm thick spacers. The gelation process was performed during 15 min, at ~ 0°C and 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 Millipore membrane (Bagyan S. et al., 2005).

Silica gel: Tetramethoxysilane (TMOS; Fluka, cat. No. 87682) was mixed with distilled water and HCl (0.1 M) in TMOS:H_2O:HCl mol ratio = $1:5:10^{-2}$, to form a clear solution and left to pre-polymerize for 24 h at 4°C (Podrazky O. et al. (2005).

The pre-polymerized **TMOS** was used for immobilization. The cell suspension (1 mL) was mixed together with 0.05 M NaOH (0.5 mL) and with pre-polymerized TMOS (0.5 mL). Than the mixture was vortexed and poured on the black Millipore membrane and an appropriate spacer ring. Subsequently, planar (disk) films with a diameter of 24 mm and with a thickness of 1.5 mm were very quickly cut out and the spacer ring was removed.

Alginate: (3.2% (w/v) sodium alginate; Sigma-Aldrich, type IV, practical grade). The sterile alginate in sterile distilled water (sterilization at 120 kPa, 120°C, 20 min) was dissolved and stored for further work at 4°C.

The **alginate/TMOS** matrix (mixture in volume ratio = 1:2) was used for immobilization. The cell suspension (1 mL) was mixed together with 0.05 M NaOH (0.5 mL), dissolved alginate (0.167 mL) and pre-polymerized TMOS (0.333 mL). Than the mixture was very quickly vortexed and poured on the black Millipore membrane and appropriate spacer ring. After gelation (within ~ 1 - 2 min) the biofilm was immersed into $CaCl_2$ solution (7 g L^{-1}) for 30 min (Podrazky O. et al. (2005). Subsequently, planar (disk) films with a diameter of 24 mm and with a thickness of 1.5 mm were cut out and the spacer ring was removed.

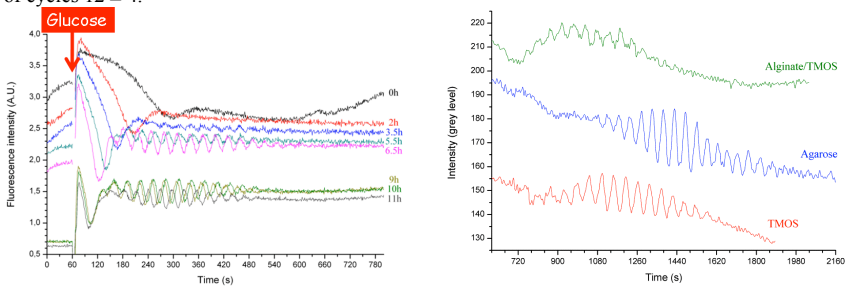
Glycolytic oscillations of immobilized cells

The biofilms of immobilized yeast cells were placed in an open spatial reactor (flowing system) as component part of the optical set up, both developed at the IEP in Magdeburg (Bagyan S. et al., 2005). Glycolytic oscillations of immobilized yeast cells were monitored by NAD(P)H fluorescence of the yeast cells with an image intensified CCD camera (Corail, Optronics, USA) and expressed as the intensity of NAD(P)H in grey level. Glycolytic oscillations of immobilized yeast cells were initiated by feeding of a solution containing 120 mM glucose and 0.2 mM acetaldehyde. The spatio-temporal dynamics of NAD(P)H fluorescence and wave propagation in the immobilized cell layers were analyzed from the recorded images and evaluated as grey levels. For analysis of the spatio-temporal oscillations, a grey level analysis was performed by averaging the grey levels of a selected image area (40 x 40 pixels out of 768 x 576 pixels) and plotting these values as a function of time.

RESULTS AND DISCUSSION

Glycolytic oscillations of yeast in cell suspensions and yeast immobilized in gel matrices

The glycolytic oscillations in cell suspensions (Fig. 1(a)) were initiated by addition of glucose (at time 60 s). First oscillations were observed when the yeast cells were starved for at least 3.5 hours. The small amplitudes of the oscillations rise with the duration of starvation. After 9 hours of starvation, the glycolytic oscillations of yeast oscillated with period of 30 ± 4 s and with the number of cycles 12 ± 4.



(a) (b)

Fig. 1: The glycolytic oscillations of the yeast *S. carlsbergensis* ATCC 9080 (10% wet (w/v)) measured: (a) in the cell suspension (different time of starvation), (b) cells immobilized in gel matrices after 9 hours of starvation.

After 9 hours of starvation, yeast cells from the starved cell suspension were immobilized. The chemical composition and preparation processes influence the type of the gel matrix. In general, the agarose gel is formed by cooling down on ice (Bagyan S. et al., 2005), whereas silica (TMOS) and alginate matrices are formed by crosslinking chemical reactions by sol-gel method (Podrazky O. et al., 2005). The glycolytic oscillations of immobilized yeast cells (Fig. 1(b)) were initiated by addition of glucose and acetaldehyde (at time 180 s). Macroscopic oscillations on the cell population level result from cell–cell synchronization via diffusion of acetaldehyde. The acetaldehyde freely permeates through the cell membrane and indirectly influences the oscillatory dynamics (Richard P. et al., 1996). The periods of the oscillations of immobilized cells were twice as large as those for cells in suspension after 9 hours starvation. They were 52 ± 9 s for alginate/TMOS, 53 ± 10 s for TMOS and 52.5 ± 7 s for agarose. The number of cycles was 12 ± 4 for the cell suspension and was only slightly influenced by immobilization reaching 15 ± 2.

The amplitudes of glycolytic oscillations of immobilized yeast cells decreased depending on the used matrices: **agarose** > **TMOS** > **alginate/TMOS**. These changes are probably caused by different diffusion coefficients of glucose and acetaldehyde trough the matrices.

Spatio-temporal dynamics

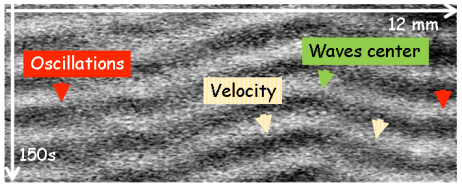
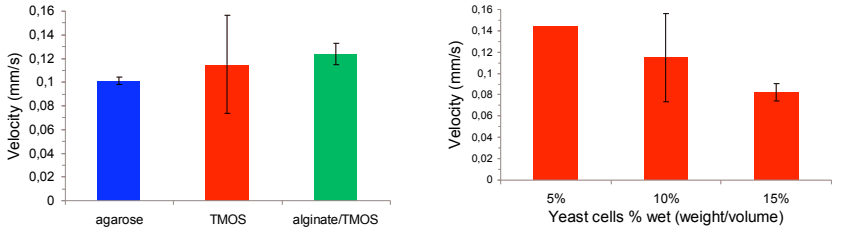


Fig. 2. displays the results of spatio-temporal dynamics of NAD(P)H waves in a time-space plot, as observed in the experiments shown in Fig. 1(b). After an induction time of 180 s, NAD(P)H waves were spontaneously generated. The NAD(P)H wave propagation depended on the type of the matrix.

Fig. 2. Time-space plot of propagating waves of immobilized yeast cells (10% wet (w/v)) in silica (TMOS) matrix. The plot was taken from the pictures of NAD(P)H intensities (in grey level) as obtained from the measurements indicated in Fig. 1(b). The bright regions correspond to increased concentrations of NAD(P)H.

The velocity of wave propagation calculated from the time-space plot (Fig. 2) depends on both, the nature of the matrix (Fig. 3(a)) and on the cell concentrations (Fig. 3(b)).



(a) (b)

Fig. 3: Velocity of wave propagation in cell suspensions of the yeast *S. carlsbergensis* ATCC 9080 immobilized in gel matrices (cell concentration: 10% wet (w/v)) (a) and yeasts immobilized in TMOS matrix (b).

CONCLUSIONS

The steady state of yeast cells is affected by the different gel matrices. Spatiotemporal dynamics and wave propagations of glycolysis were influenced by the properties of the different gel matrices. Silica matrix (TMOS) is microporous in contrast to macroporous agarose and may have greater effects on the diffusion of external substances.

REFERENCES

Bagyan S. et al. (2005) *Glycolytic oscillations and waves in an open spatial reactor: Impact of feedback regulation of phosphofructokinase*. Biophysical Chemistry 116, 67–76.
Betz A. (1966) *Metabolic flux in Yeast Cells with Oscillatory Controlled Glycolysis*. Physiologia Plantarium 19, 1049-1054.
Danø S. et al. (1999) *Sustained oscillations in living cells*. Nature 402, 320–322.
Doran M.P. et al. (1987) *Effects of Immobilization on the Nature of Glycolytic Oscillations in Yeast*. Biotechnology and Bioengineering 29, 892–897.
Gavlasova P. et al. (2008) *Whole cell biosensor for polychlorinated biphenyl analysis based on optical detection*. International Biodeterioration & Biodegradation 62, 304–312.
Ghosh A. et al. (1964) *Oscillations of glycolytic intermediates in yeast cells*. Biochemical Biophysical Research Communications 16, 174–181.
Hess B. et al. (1968) *Mechanism of glycolytic oscillation in yeast, 1. Aerobic and anaerobic growth conditions for obtaining glycolytic oscillations*. Hoppe-Seylers Zeitschrift fur Physiologishe Chemie 349, 1567–1574.
Naessens M. et al. (2000) *Fiber Optic Biosensor Using Chlorella vulgaris for Determination of Toxic Compounds*. Ecotoxicology and Environmental Safety 46, 181–185.
Podrazky O. et al. (2005) *Determination of concentration of living immobilized yeast cells by fluorescence spectroscopy*. Sensors and Actuators B-Chemical 107, 126–134.
Poulsen A.K. et al. (2007) *Single cell studies and simulation of cell-cell interactions using oscillating glycolysis in yeast cells*. Biophysical chemistry 125, 275–280.
Preininger C. et al. (1994) *Optical Fiber Sensor for Biological Oxygen Demand*. Analytical Chemistry 66, 1841 – 1846.
Richard P. et al. (1996) *Acetaldehyde mediates the synchronization of sustained glycolytic oscillations in populations of yeast cells*. European Journal of Biochemistry 235, 238–241.
Richard P. (2003) *The rhythm of yeast*. FEMS Microbiology Rewiews 27, 547–557.
Trogl J. et al. (2005) *Selectivity of whole cell optical biosensor with immobilized bioreporter Pseudomonas fluorescens HK44*. Sensors and Actuators 107, 98–103.

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