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The Effect of Pulse Electric Field on Accumulation of Selenium in Cells of *Saccharomyces cerevisiae*

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Abstract Cultures of *Saccharomyces cerevisiae* were subjected to the effect of PEF (pulse electric field) and a source of selenium. The culture period after which yeast cells were subjected to PEF treatment was optimized, as was the duration of the exposure. Optimization of the nutrient medium composition in *S. cerevisiae* cultures resulted in an over 1.8-fold increase in selenium accumulation with relation to cultures on the initial substrate. Optimization of the pH value and of culture duration resulted in selenium accumulation increase by approximately 78%. A significant correlation was found between the accumulation of selenium in yeast cells and its concentration in the culture substrate. The highest accumulation of selenium in the biomass of yeast, approx. 240 μg/g d.m., was obtained after 15-min exposure to PEF on a 20-h culture. An approx. 50% higher content of selenium in cells was recorded, as compared with the control culture without the application of PEF.

Keywords: Selenium, biomass, *Saccharomyces cerevisiae*, pulse electric field

Selenium is one of the more important microelements necessary for correct functioning of living organisms. Its role is largely related to the activity of selenium-dependent glutathione peroxidase [7, 13, 21, 30]. Selenium-enriched preparations are used in neoplastic prophylaxis [31, 36]. In oral preparations, selenium occurs in the form of sodium selenite, methionine selenide, and selenium yeast, whose physiological activity is well documented [14, 20, 25, 38]. Selenium concentration in an organism increases the fastest when the diet includes selenium yeast; the inorganic forms are assimilated more slowly. Selenium building into yeast cells significantly reduces the risk of intoxication of the organism [6]. Microbial cells are capable of selenium accumulation in the form of organic bonds and inorganic compounds. Two selenium binding mechanisms are known: as extracellular accumulation, through active groups of biopolymers of the cell wall-membrane system, and intracellular accumulation combined with ion transport through the membrane into the cell. The cell wall binds approximately 20% of selenium, the remaining part being contained within the protoplasts [5]. So far, *S. cerevisiae* enriched in selenium were grown on nutrient substrates lean in sulfur compounds, with admixtures of inorganic salts of selenium [9, 10, 33].

One of the relatively easy and at the same time nontoxic and inexpensive methods for the introduction of specific macromolecules to the cytoplasm is electroporation by PEF processing. PEF treatment is a new nonthermal technology used mainly for the inactivation of microorganisms [3, 11, 16, 28, 37, 39]. It is generally accepted that PEF exerts its effect by causing the formation of pores in the membrane of treated cells [2–4, 15, 34, 35]. In a cell subjected to PEF, induced transmembrane voltage leads to the formation of pores in the membrane and to an increase in its permeability. It may reach a level permiting cell penetration by extracellular molecules. Such a state of membrane permeability for macromolecules following electroporation may persist for a period from several minutes to more than 10 h, depending on the type and size of cells, electric field intensity, pulse duration, temperature, and chemical composition of the medium. After cessation of the PEF effect, there may occur a reversible process of self-sealing of the formed pores, with retention of foreign molecules [12, 29, 32]. If the applied intensity of electric field attains or exceeds the critical value, then a large number of holes of considerable dimensions are formed under such conditions, which leads to irreversible mechanical damage to the cell [17, 40].

The aim of the present work was to determine the impact of an electric field on the growth of *S. cerevisiae* and incorporation of selenium into cells at different selenium concentrations in the medium.

We assumed that the exposition time to PEF and the time interval of sodium selenite application to culturing medium

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as well as the harvest time of yeast affect the accumulation of selenium in cells.

MATERIALS AND METHODS

Optimization of Substrate Composition and *S. cerevisiae* Culture Parameters

The study was performed using production yeast *Saccharomyces cerevisiae* 11 B_1 from the yeast factory at Kunickiego Street in Lublin.

The base liquid substrate had the following composition (g/l): saccharose (20.0), NH_4Cl (3.2), KH_2PO_4 (2.5), $MgCl_2 \cdot 6H_2O$ (1.5), Na_2SO_4 (2.0), yeast extract (YE) (5.0), and 40 ml of unhopped wort. Substrate sterilization was performed in an autoclave at 113°C for 20 min. The base culture medium, with the above composition, was modified as follows:

a) substrate for determinations of optimum content of carbon: base medium plus an admixture of saccharose in the following amounts (g/l of the medium): 20, 40, 60, 70, 80, 90, 100, 120.

b) substrate for determinations of the effect of various sources of carbon: base medium plus an admixture of glucose (instead of saccharose) in the following amounts (g/l of the medium): 20, 40, 60, 70, 80, 90, 100, 120.

c) substrate for determinations of optimum content of nitrogen source: base medium with a fixed content of glucose, plus an admixture of NH_4Cl in the following amounts (g/l): 2, 3, 4, 5, 6, 7.5, 9,10.

d) substrate for determinations of optimum dosage of potassium: base medium with a fixed content of glucose and NH_4Cl plus an admixture of KH_2PO_4 in the following amounts (g/l): 1, 1.5, 2.5, 3, 4, 5.5, 7, 8.

e) substrate for determinations of optimum content of magnesium source: base medium with fixed content of glucose, NH_4Cl, and KH_2PO_4 as above, plus $MgCl_2 \cdot 6H_2O$ in the following amounts (g/l): 1, 1.3, 1.5, 1.7, 2, 3.2, 4, 5.

f) substrate for determinations of optimum content of sulfur source: base medium with fixed content of glucose, NH_4Cl, KH_2PO_4, and $MgCl_2 \cdot 6H_2O$ as above, plus Na_2SO_4 in the following amounts (g/l): 1, 2, 3, 4.5, 6, 7, 9, 10.

Liquid medium for optimization of pH, duration of culture, and selenium concentration in the substrate had the following composition (g/l): glucose (70.0), NH_4Cl (7.5), KH_2PO_4 (2.5), $MgCl_2 \cdot 6H_2O$ (2.0), Na_2SO_4 (2.0), yeast extract (YE) (5.0), and 40 ml of unhopped wort. Optimization of the pH value of culture worts covered the range from 3.5 to 7, with increment of 0.5 unit. Culture duration was optimized within periods of 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, and 72 h, at fixed nutrient medium composition and pH value.

In another experiment, the effect of selenium concentration in nutrient medium on its accumulation in cells was demonstrated. Cultures of *S. cerevisiae* were grown at selenium concentration in the nutrient medium at 1, 2, 3, 4, 6, 8 (μg/ml). Sodium selenite was added in five portions in the course of the culture.

Determination of Yeast Cell Viability

In order to determine the viability of yeast cells, 2 ml of post-culturing liquid was centrifuged and then decanted. The remaining yeast was washed with distilled water, decanted, and topped up with water to the initial volume of 2 ml. After thorough stirring, one drop of the solution was placed in a Thom chamber, adding to the sample a drop of 0.01% solution of methylene blue to dye dead cells. In the resultant preparation, a count was taken of living cells (non-dyed) and of dead cells (dyed blue). The percentage of dead cells was a mean value for 16 fields calculated according to the formula:

% of dead cells=(number of dead cells /sum of dead and living cells) 100%

Electroporation of Cell Membranes of *S. cerevisiae*

Electroporation of cell membranes of *S. cerevisiae* was performed in accordance with the schematic presented in Fig. 1.

The study objects in the experiments were as follows: (1) yeast culture to which 4 μg Se/ml of nutrient medium was added in five time-stepped partial doses, first before the start of the culture, the next ones after 8, 12, 16, 20 h, and the culture was treated with PEF after 8 h from culture start; (2) yeast culture to which selenium was added in five time-stepped portions as above, and the culture was treated with PEF after 12 h from culture start; (3) yeast culture to which selenium was added in five time-stepped portions as above and which was PEF treated after 16 h; (4) yeast culture to which selenium was added at time intervals as above and which was PEF treated after 20 h; (5) yeast

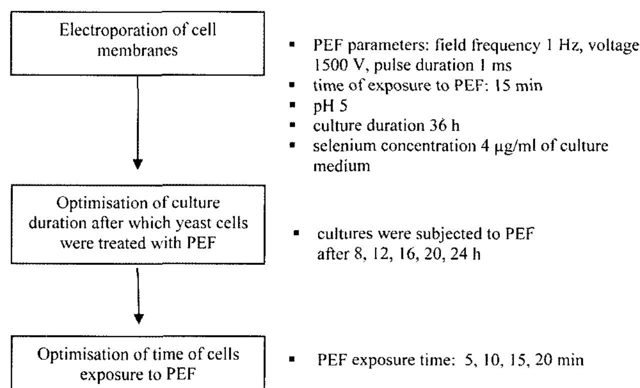

Fig. 1. Electroporation of yeast cell membranes.

culture to which selenium was added at time intervals as above and which was PEF treated after 24 h. Control cultures: (K1) yeast culture with no selenium in the culture medium, without PEF treatment; (K2) yeast culture with the full dose of 4 μg Se/ml in the culture medium at specified concentration, added to the medium before the start of the culture, without PEF treatment; (K4) yeast culture to which selenium was added in five partial doses at time intervals as above and which was treated with PEF after 8, 12, 16, 20 h of growth. When determining the optimum culture duration after which yeast cells were treated with PEF, an additional control culture (K3), was used, to which the full dose of selenium was added prior to the start of the culture and which was treated with PEF after 20 h of yeast cell multiplication.

In order to optimize the duration of exposure of *S. cerevisiae* cells to the effect of PEF, the experimental objects 1 to 5 were treated with PEF for periods of 5, 10, 15, or 20 min.

Culturing Process and Analytical Procedure

Preparation of inoculation material: Yeast was passaged several times for agar swaths and grown for 48 h in a thermostat at 30°C. Next, the inoculate was prepared. Cells from a single swath were used to inoculate 150 ml of sterile nutrient medium in an Erlenmeyer flask. Cultures were grown on a rotary shaker with water bath, at amplitude 4 and at 220 rpm for 48 h at 30°C. The post-culturing fluid was removed, and the remaining washed biomass of cells from three Erlenmeyer flasks was mixed with sterile water to a final volume of 300 ml. The inoculate prepared in this manner was used for inoculation of depth cultures that were grown in conical flasks of 500 ml in volume, each containing 100 ml of nutrient medium inoculated with 10 ml of the inoculate. The culturing conditions were identical to those of the inoculate preparation. After the culturing was complete, the mycelia were centrifuged, washed several times with distilled water, and then lyophilized in a Labconco freeze dryer (Model 64132, Kansas City, MO, U.S.A.).

Method for the Determination of Selenium Concentration

Mineralization of yeast for the determination of selenium concentration with the method of atomic absorption spectrophotometry was conducted as follows: portions of 100 mg of lyophilized selenium yeast were weighed into glass thimbles, 5 ml of HNO_3-$HClO_4$ (3+1 v/v) mixture was added, and the sample mixture was left overnight. The following day, the samples were heated in a heating block at 50°C for 1 h, at 70°C for 6 h, and at 125°C for 12 h. After cooling, solutions for determinations with the method of electrothermal atomic absorption spectrometry (ETAAS) were transferred to measuring flasks of 25 ml in volume and topped up with distilled water.

Selenium was determined with the method of atomic absorption spectrophotometry, with the flameless technique, using a graphite cuvette in a Varian Spectra AA-880 apparatus.

In order to find significant differences between particular groups, the t-test was applied to compare independent samples in pairs, and variance analysis (ANOVA) was used for more than two groups. Statistical processing of results was performed using Statistica 6.0 software.

Preparation Procedure for Electron Microscopy Examinations in a Transmission Electron Microscope (TEM)

For examinations in the transmission electron microscope, *Saccharomyces cerevisiae* yeast was used that originated from a control culture without exposure to PEF, as well as yeast from a culture treated with PEF for 15 min after 20 h from culture start.

Samples for the examinations were prepared as follows: The yeast was fixated with a 4% solution of glutaraldehyde in a nutrient medium of pH 5. Next, the yeast suspension was hardened for 2 h in a 1% solution of agarose, and only then was the yeast pellet subjected to final hardening.

The preparations were subjected to contrast enhancement in a 1% solution of OsO_4 in 0.1 M cacodylate buffer with pH 7.4 for 2 h at 4°C. After washing with the same buffer, and then with distilled water, the preparations were again subjected to contrast enhancement in a 0.5% water solution uranyl acetate for 2 h at room temperature. The preparations were dehydrated in alcohol solutions and the ethyl alcohol was next replaced with propylene oxide. In turn, propylene oxide was replaced with a resin, with increasing resin concentrations in propylene oxide. The preparations were hardened in Spurr Low Viscosity resin and left for 12 h at 70°C to polymerize. Ultrathin preparations were dyed with an 8% solution of uranyl acetate in a 0.5% solution of acetic acid for 45 min, and after washing with distilled water and drying, additionally dyed for 10 min with Reynold's lead citrate. Electron microscope documentation was performed by means of a Tesla BS-500 microscope.

RESULTS

Optimization of Culture Medium Composition and *S. cerevisiae* Culture Parameters

The performed study showed that from the two tested sources of carbon (glucose or saccharose) included in the composition of the nutrient medium, the application of glucose resulted in a greater accumulation of selenium, by an average of approximately 27% (Fig. 2). With the optimum content of glucose (70 g/l), the maximum accumulation of selenium was recorded in cells of *S. cerevisiae*, at about 40 μg/g d.m.

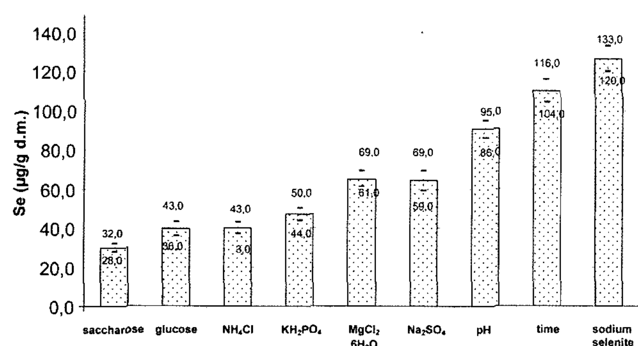

Fig. 2. Effect of optimum composition of nutrient medium and culture parameters on selenium accumulation in cells of *Saccharomyces cerevisiae.*

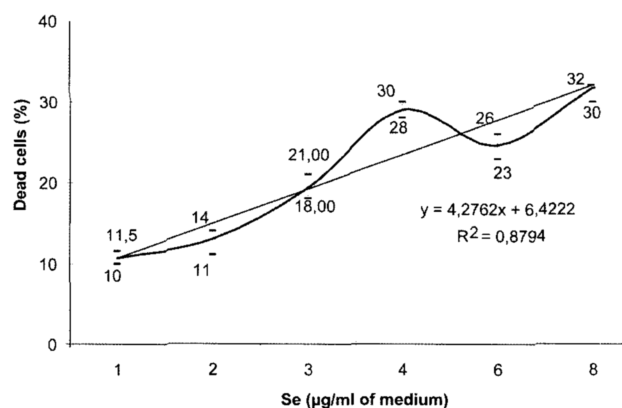

Fig. 3. Effect of selenium concentration in nutrient medium on the viability of cells of *S. cerevisiae.*

The results indicate a significant effect of the concentration of all the components of the nutrient medium on the accumulation of selenium in yeast cells (Table 1). The exceptions were the sources of nitrogen and of sulfur (Fig. 2). At the optimum content of nitrogen source (7.5 g/l), the maximum accumulation of selenium was recorded, at about 40 µg/g d.m. Optimization of the source of nitrogen, however, did not result in any statistically significant increase in selenium accumulation in yeast, as compared with the culture in which the carbon source was determined (Table 1). Optimization of potassium and magnesium sources as successive components of the nutrient medium resulted in an over 60% increase in selenium accumulation, up to approximately 65 µg/g d.m. with relation to the culture in which nitrogen source was determined (Fig. 2).

With the optimum content of Na_2SO_4 in the nutrient medium, 2 g/l, approx. 60 µg/g d.m. of selenium was determined in the yeast. Optimization of the sulfur source in the nutrient medium had no effect on the accumulation of selenium in cells.

Optimum composition of the nutrient medium in terms of the sources of carbon, potassium, magnesium, and sulfur resulted in an over 80% increase in selenium accumulation (Fig. 2).

Further optimization of the pH value and culture duration (Fig. 2) resulted in an increase in selenium accumulation by about 78%. At pH from 3.5 to 5, an increase of about 51% was recorded in selenium accumulation in yeast biomass. At the optimum level of pH 5, the maximum selenium accumulation was found, at about 91 µg/g d.m. Further increase in pH value was not conducive to selenium accumulation (Table 1).

Studies of selenium accumulation with relation to culture duration showed that the greatest accumulation, approx. 110 µg/g d.m., was recorded in cells originating from the 36-h culture.

Another experimental task was performed to demonstrate the effect of selenium concentration in the nutrient medium on its accumulation in cells of *S. cerevisiae* (Fig. 2). At the optimum concentration of selenium in the nutrient medium, 4 µg/ml, its maximum accumulation was recorded, approx. 127 µg/g d.m. Determination of the optimum concentration of selenium in the nutrient medium resulted in an increase in its accumulation by about 15% with relation to cultures grown at previously adopted parameters (Table 1).

Effect of Selenium Concentration on Viability of Cells of *S. cerevisiae*

Subsequent experiments involved measurement of the viability of yeast cells. The effect of selenium concentration in the nutrient medium on yeast cell viability is presented in Fig. 3.

The performed studies indicate that at selenium concentration of 1 µg/ml, the share of dead cells in a culture was about 11%. Within the selenium concentration range from 2 to 4 µg/ml, the number of dead cells was observed

Table 1. Selenium accumulation in *S. cerevisiae* after step-by-step optimization of medium components and culture parameters.

Medium components and culture parameters	Saccharose	Glucose	NH_4Cl	KH_2PO_4	$MgCl_2 \cdot 6H_2O$	Na_2SO_4	pH	Time	Sodium selenite
M	30	40 a	40 a	47	65 b	65 b	91	109,5	126,5
SD	1,83	2,94	2,58	2,94	3,65	4,55	4,24	5,92	6,95

M, mean values; SD, standard deviation; mean values denoted with the same letter (a, b) are not significantly different statistically at $P \geq 0.05$; n=6; all other mean values significantly differ statistically at $P \leq 0.05$; n=6.

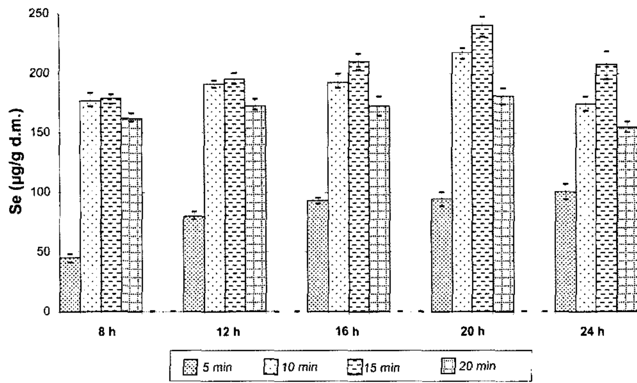

Fig. 4. Effect of PEF exposure time and duration of culture on the content of selenium in cells of *S. cerevisiae*.

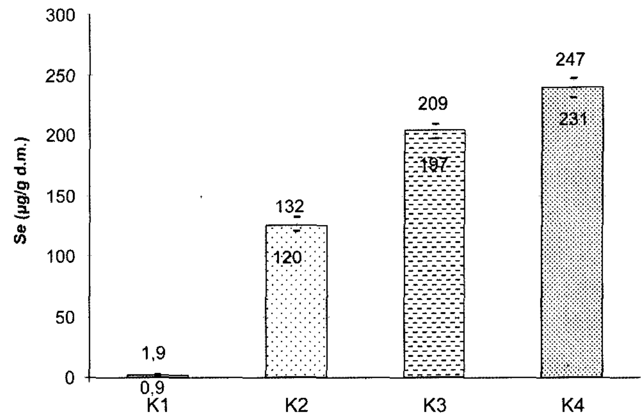

Fig. 5. Selenium concentration in cells of *S. cerevisiae*. Control cultures: (K1) no Se and no PEF; (K2) full dose of selenium, no PEF; (K3) full dose of selenium and PEF treatment when culture 20 h old; (K4) full dose of selenium and PEF treatment after 8, 12, 16, 20 h of culture growth.

to double. Further increase in selenium concentration had no significant effect on the viability of yeast cells.

Electroporation of Yeast Cell Membranes

Further experiments were aimed at determination of the optimum period of yeast culture after which the cells were treated with PEF, and at the selection of optimum duration of exposure to PEF (Fig. 4).

Under the adopted culture conditions, maximum accumulation of selenium in yeast, approx. 240 µg/g d.m., was observed after PEF treatment of a 20-h culture. There was an increase in selenium content by approx. 37% with relation to cells treated with PEF after 8 hours of culture growth (Fig. 4).

To better illustrate the effect of PEF on selenium accumulation in yeast, control cultures were maintained at the same time (Fig. 5). In the control culture K1, to which no selenium was added and which was not subjected to treatment with PEF, only trace amounts of selenium were observed, below 1 µg/g d.m. A significant increase (Table 2) in selenium accumulation in cells was recorded in the control culture K2, which received the full dose of selenium but was not treated with PEF (Fig. 5). At the same time, the level of selenium accumulation obtained in K2 was lower than in those cultures that were subjected to PEF treatment. PEF treatment of the control culture K3 resulted in a statistically significant increase (Table 2) in selenium accumulation as compared with K2. It was observed that Se accumulation in yeast cells exceeded 200 µg/g d.m., when the whole dose of Se was added to the medium prior to the culture start that was PEF treated after 20 h for 15 min (K3, Fig. 5). The amount of selenium accumulated differed statistically significantly as compared with cells grown in medium supplemented with five doses of sodium selenite, at the same PEF processing parameters (Fig. 4). No statistically significant differences (Table 2) in selenium accumulation were observed in yeast from the control culture (K4) in which cells were subjected to PEF treatment fours times, after 8, 12, 16, and 20 h, as compared with the culture that was PEF treated once, for 15 min, after 20 h from the culture start and to which selenium was added in five partial doses. Multiple exposure of yeast cultures to PEF was not conducive to increased accumulation of selenium in yeast cells, neither was the addition of the full dose of selenium in several partial doses in the course of the culture duration.

Table 2. Selenium accumulation in *S. cerevisiae* cells in particular groups.

Groups	8 h				12 h				16 h			
min	5	10	15	20	5	10	15	20	5	10	15	20
M	45,0	177,0	178,6 j	162,0	79,7	190,7	195,0 i	172,7	93,0	192,0	210,0 h	172,7
SD	3,61	5,57	4,16	3,61	3,06	3,51	4,58	4,73	2,65	6,24	6,56	8,08

Groups	20 h				24 h				K1	K2	K3	K4
min	5	10	15	20	5	10	15	20				
M	94,7 d	217,7 d,e	240,0 e,f,g,h,i,j,*	181,3 f	101,0	174,7	208,0 g	155,0	1,5 a	125,0 a,b	204,0 b,c	240,0 c,*
SD	6,11	4,93	8,19	6,66	6,24	6,11	11,79	4,58	0,53	6,24	6,24	8,19

M, mean values; SD, standard deviation; mean values denoted with the same letter (a, b, c, d, e ...) are significantly different statistically at $P \leq 0.05$; $n=6$. Mean values denoted with an asterisk (*) do not differ statistically significantly at $P \geq 0.05$; $n=6$.

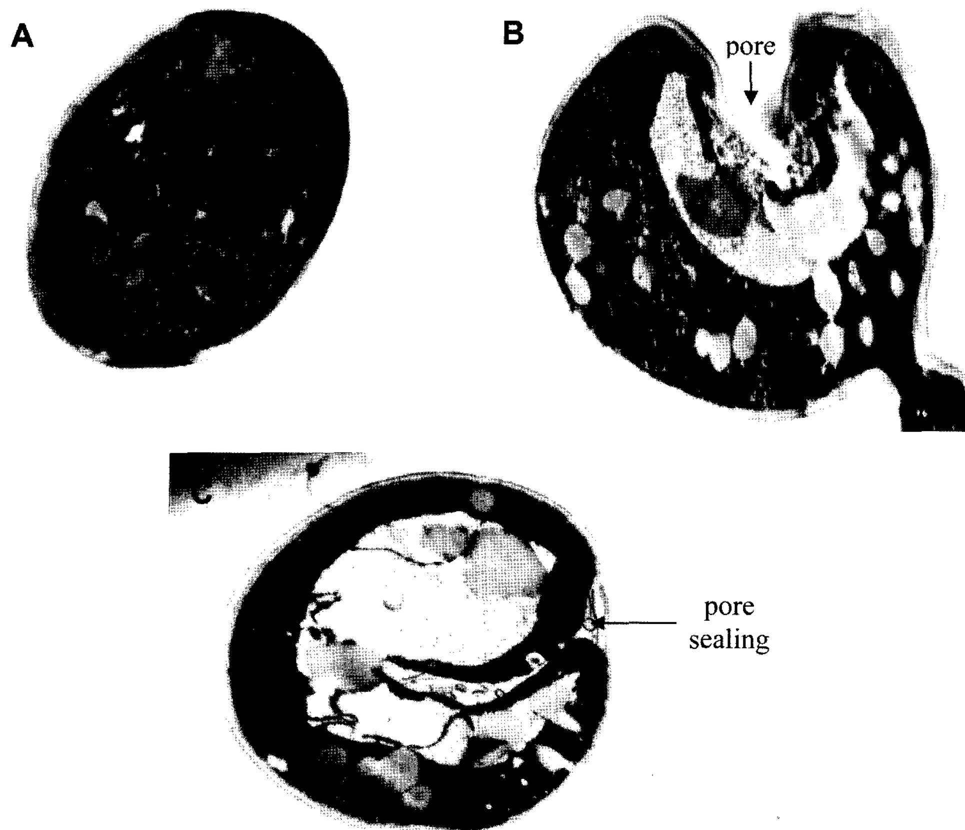

Fig. 6. Cells of *S. cerevisiae* from a 36-h culture: (**A**) control without PEF treatment; (**B–C**) treated with PEF. Photographs made in a transmission electron microscope. Rate of magnifications applied, ×15,000.

Determination of the optimum PEF exposure time for a 20-h culture resulted in an increase of selenium content in yeast cell biomass. After 15-min PEF exposure, the highest selenium accumulation in the cells was obtained (approx. 240 μg/g d.m.). It was higher by approx. 166% or 28% relative to 5- and 20-min treatments of yeast culture with PEF (Fig. 4).

Optimization of *S. cerevisiae* culture duration and PEF exposure time resulted in an over 2-fold increase in selenium accumulation relative to the control culture without the application of PEF.

Results of Electron Microscopy Examinations

In order to confirm the effect of PEF on cells of *S. cerevisiae*, photographs were made of suitable preparations.

In the photograph in Fig. 6A, taken in a TEM, the yeast cell originating from the control culture has a typical oval shape, with visible cell structures such as the nucleus, vacuoles, mitochondria, and ribosomes. Figs. 6B and 6C present a cell of *S. cerevisiae* from a culture subjected to PEF treatment. Visible in the photographs are the cell structures: nucleus, mitochondria, vacuoles, and ribosomes. Fig. 6B provides a record of the process of yeast cell multiplication through gemmation. In both photographs, the regular oval shape of the cell is disturbed and changes are visible in the form of pores. The pores are the effect of the cultures having been treated with PEF, as that was the only parameter that differentiated the culture from the control (Fig. 6A). Fig. 6C provides a record of the sealing of a pore, as the process of pore formation is a reversible phenomenon when the PEF parameters are properly selected experimentally.

DISCUSSION

Results of experiments on selenium accumulation in *S. cerevisiae* indicate that the optimization of selenium concentration in the nutrient medium is a necessity [9, 26, 33]. The incorporation of selenium by the yeast cells depends on the composition of the growing media, (*e.g.*, different source and concentration of carbon and Se), culture conditions like time (phase of growth) of selenium addition, form of Se as well as harvest time. Culture media used contain sodium selenite or sodium selenate with minimal sulfur concentration [8, 18, 27, 33]. In our study,

the optimum Se concentration was 4 μg/ml. In yeast species *Rhodotorula rubra* and *Kluyveromyces marxianus*, different optima were determined as 15 μg/ml and 14 μg/ml, respectively [23, 24].

The method of sodium selenite addition to the nutrient medium also had an effect on the accumulation of selenium in yeast cells. In cultures of *S. cerevisiae*, greater accumulation was obtained when sodium selenite was added in five partial doses in the course of culturing. In cultures of *R. rubra* [24] and *K. marxianus* [23], no statistically significant differences in selenium accumulation were observed after periodical introduction of the full dose of selenium into the nutrient medium nor after the full dose of selenium was added prior to the start of the culture.

Suhajda *et al.* [33], in cultures of *S. cerevisiae,* also used sodium selenite as the source of selenium in the nutrient medium. They obtained the highest accumulation of selenium in cells (2,050 μg/g d.m.) with the addition of 30 μg/ml of sodium selenite in six partial doses spread evenly throughout the exponential phase of yeast growth. They observed that the addition of sodium selenite after the phase of logarithmic growth results in lower accumulation of selenium (1,500 μg/g d.m.). Similar conclusions were reached by Achremowicz and Podgórska [1] in a study on enriching *S. cerevisiae* with selenium. They determined the optimum dose of sodium selenite for addition to the nutrient medium (30 μg/ml) to avoid the inhibiting effect of selenium on yeast growth. Sodium selenite was administered in 6 doses of 5 μg/ml each, every hour of the exponential phase of growth. Under those conditions, the authors obtained the highest accumulation of selenium of approximately 1,500 μg/g d.m. Kaur and Bansal [18], observed decreasing *S. cerevisiae* culture cells at increasing of Se concentration as sodium selenite, from 19.9 μM to 57 μM. The Se content in yeast cells was increased about two-fold at a higher dose of selenite.

Demirci and Pometto [9] optimized the composition of the nutrient medium in order to obtain maximum accumulation of selenium in cultures of *S. cerevisiae.* They used sodium selenite and selenate as the source of selenium in the nutrient medium, at the minimum predetermined concentration of sulfur. In cultures in which sodium selenite was used, selenium accumulation in cells was obtained at the level of 1,904 μg/g d.m., whereas in those with an admixture of selenate, it was approx. 687 μg/g d.m.

Korhola *et al.* [19], in a study on *S. cerevisiae,* demonstrated that the yeast accumulated selenium in the amount of 500 μg Se/g d.m. Nagodawithana and Gutmanis [22], conducting studies on selenium-organic compounds in *S. cerevisiae* yeast, obtained the level of 1,000 μg Se/g d.m. in yeast grown on a nutrient medium in which sodium selenite was used as the selenium source. The application of various levels of selenium concentration in the nutrient media was - probably - the reason for the varied accumulation of selenium in yeast cells.

Membrane electroporation caused by electric field processing of *E. coli*, *L. innocua*, and *S. cerevisiae* was studied by Aronsson *et al.* [3]. Electrical field strength and pulse duration influenced membrane electroporation of all organisms of which *S. cerevisiae* was the most PEF sensitive, although very little or no inactivation was observed.

In the study, reported herein, a significant effect of PEF on selenium accumulation in cells of *S. cerevisiae* was demonstrated. Optimization of culturing time and of the duration of exposure of *S. cerevisiae* to the effect of PEF resulted in more than two-fold increase in selenium accumulation relative to control culture without PEF treatment. Similar results were obtained for *K. marxianus* [23] and for *R. rubra* [24].

S. cerevisiae proved to be the most resistant to the effect of electric field, where the optimum time of exposure to PEF was the longest (15 min). The most susceptible to the effect of electric field were cells of *K. marxianus* [23] and of *R. rubra* [24]; for those kinds of yeast, the optimum times of exposure were shorter, at 3 and 10 min, respectively.

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