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Behavior of yeast cells in aqueous suspension affected by pulsed electric field

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Abstract

This work discusses pulsed electric fields (PEF) induced effects in treatment of aqueous suspensions of concentrated yeast cells (*S. cerevisiae*). The PEF treatment was done using pulses of near-rectangular shape, electric field strength was within $E = 2$–5 kV/cm and the total time of treatment was $t_{PEF} = 10^{-4}$–0.1 s. The concentration of aqueous yeast suspensions was in the interval of $C_Y = 0$–22 (wt%), where 1% concentration corresponds to the cellular density of 2×10^8 cells/mL. Triton X-100 was used for studying non-ionic surfactant additive effects. The electric current peak value I was measured during each pulse application, and from these data the electrical conductivity σ was estimated. The PEF-induced damage results in increase of σ with t_{PEF} increasing and attains its saturation level $\sigma \approx \sigma_{max}$ at long time of PEF treatment. The value of σ_{max} reflects the efficiency of damage. The reduced efficiency of damage at suspension volume concentration higher than $\varphi_Y \approx 32$ vol% is explained by the percolation phenomenon in the randomly packed suspension of near-spherical cells. The higher cytoplasmic ions leakage was observed in presence of surfactant. Experiments were carried out in the static and continuous flow treatment chambers in order to reveal the effects of mixing in PEF-treatment efficiency. A noticeable aggregation of the yeast cells was observed in the static flow chamber during the PEF treatment, while aggregation was not so pronounced in the continuous flow chamber. The nature of the enhanced aggregation under the PEF treatment was revealed by the ζ-potential measurements: these data demonstrate different ζ-potential signs for alive and dead cells. The effect of the electric field strength on the PEF-induced extraction of the intracellular components of *S. cerevisiae* is discussed.

Keywords: Electric fields; Aqueous suspensions; Electrical conductivity; Percolation; *Saccharomyces cerevisiae*; Yeast; Non-ionic surfactant; Triton X-100

1. Introduction

The electric field application to colloidal suspensions generates a lot of interesting effects, which result in drastic changes of rheological, optical and electromagnetic properties of systems [1–6]. The external electric field polarizes the dispersed particles, enhances their attraction and aggregation [7–9], increases migration of the particles and the surrounding ions [10] and may cause deformation of the fluid particles [11].

The electric fields can provoke a considerable leakage of cytoplasmic ions in colloidal biosuspensions, which affects ionic concentration of the medium and its electrical conductivity [12–14]. It was shown that application of the pulsed electric fields (PEF) with a typical field strength E of 5–50 kV/cm and microsecond duration cause creation and growth of pores (electroporation) and the damage of the cell membranes. The electroporation theory describes the mechanism of the creation and growth of pores in the membranes [15]. In biosuspensions, the electric field is selectively concentrated on membranes, and for the idealized spherical shape of cells the transmembrane potential u_m is defined by the following relation [16]:

$$u_m = 0.75 d_c E \cos\theta, \tag{1}$$

where d_c is the cell diameter and θ is the angle between the external field E direction and the radius-vector on the membrane surface.

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The critical potential of the membrane damage is of order $u_m \sim 0.2$–1 V [15–18]. A high electric field can also cause the local overheating of membranes [19] and very fast transmembrane electroosmotic flow through the cell walls [20]. Electroporation efficiency is very sensitive to distribution of the electrical fields on a cell surface. This distribution may be influenced by the inter-cells aggregation, cell-on-surface adhesion and formation of biofilms, which are important for biological suspensions [21–25].

There are examples of PEF application for electrofusion of cells [26] and facilitation of the rapid transport of nanoparticles across the cell wall [27]. An increasing interest exists in the biotechnological PEF applications for the colloidal bioorganisms inactivation [28] and for the enhancement of proteins and other intracellular components extraction from biocells [29].

This work deals with PEF-induced effects in colloidal aqueous biosuspension of *S. cerevisiae* (brewing yeast). This classical biosuspension shows rather complex properties [23]. The nature of colloidal stability and main interaction forces between yeast cells are still under discussion [30–32]. *S. cerevisiae* cells show a strong tendency of aggregation even in the diluted suspensions (at volume concentration of $\varphi \approx 1\%$) [14,33]. That reflects the importance of the biopolymer bridging forces, related to the accumulation of specific glycoproteins on the surfaces of the cells [23]. However, in very diluted suspensions ($\varphi \approx 0.01\%$) the aggregations are suppressed even in the isoelectric point IEP (at pH 2.5). That evidences the importance of the additional repulsive hydration energy existing between *S. cerevisiae* cells [31,32].

The concentration of the cells and the state of their aggregation may contribute to PEF-induced effects in colloidal suspensions of *S. cerevisiae*. Some authors observed the increment of damage efficiency with the increment of *S. cerevisiae* cells concentration [34], others reported the opposite effect [35–37]. The mechanism of this effect is still yet unclear. The contradictions in existing experimental data can be explained by the influence of many factors: the treatment protocol (electric field strength E, wave forms, pulse duration t_i, total time of treatment t_{PEF}), the temperature T, the differences among treatment chambers, the effects of electrolytes and surfactant additives in treated biocolloidal systems. At moderate fields ($E < 7.5$ kV/cm) the incomplete damage was reported for suspensions of *S. cerevisiae* cells [14,38,39]. The effect of incomplete damage at moderate fields can be explained in part by the formation of low-conductive cores (consisting of damaged cells envelops) near the surface of intact cells inside the cells flocs [14]. To achieve a higher disintegration degree (>99.9%) of the *S. cerevisiae* cells, the high electrical field strengthes were commonly used (10–50 kV/cm) [35,40,41]. The relations between clusters formation and PEF-induced damage efficiency were explained by two different speculations. In the first speculation it was supposed that the electric fields can enhance the inter-cells attraction and produce the cells "pearl chain." That results in the increasing of the "equivalent cell" size with a larger volume and leads to the enhancing of PEF-damage efficiency [42]. In according to the second speculation, the cluster formation of yeast cells can result in arising of the protection mechanism, when undamaged cells can be hidden in low electric field sites [37]. The latter hypothesis is supported by the experimental data on increasing of damage efficiency by strong mixing of *S. cerevisiae* suspensions [14,41].

The purpose of this study was to investigate the effects of the concentration of cells and the state of their aggregation on the PEF- induced effects in biocolloidal suspensions of *S. cerevisiae*. The electrical conductivity measurements during the PEF-treatment of *S. cerevisiae* suspensions were used to monitor the extent of the damage of cells in the electric field strength interval of $E = 2$–5 kV/cm. The treatments in static and continuous flow treatment chambers were compared in order to reveal the effects of mixing on the PEF-treatment efficiency. The effects of non-ionic surfactant additives on the aggregation and the PEF-induced lethality were also discussed.

2. Experimental

2.1. Yeast cells

Yeast *S. cerevisiae* cells (Briochin, ALSA, Bestfoods, France) were used throughout this study. The commercial dry powder consists of the rod-shaped particles (Fig. 1a) and contains 70% of yeasts and 30% of additives (electrolytes and

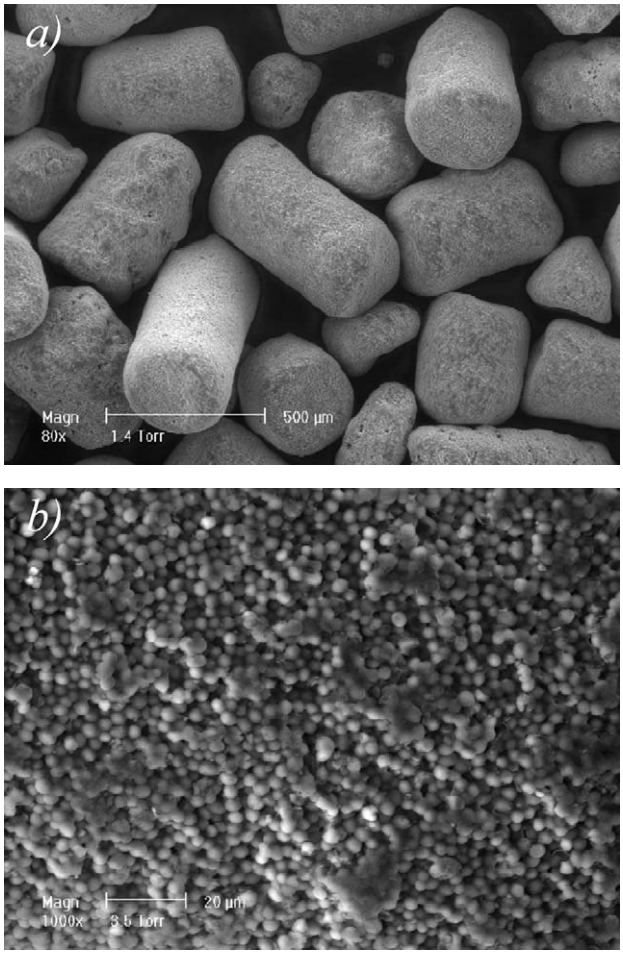

Fig. 1. ESEM images of the commercial dry powder (a) and concentrated (≈24%, w/w, concentration of yeast) aqueous suspension (b) of *S. cerevisiae* cells.