Morphological Study of *Saccharomyces Cerevisiae* Cells Treated With Magnetic Fluid

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***Abstract*—This paper shows that *Saccharomyces cerevisiae* efficiently interact with magnetite-based ionic magnetic fluid, leading to the formation of magnetically labeled cells which could be easily separated from the system using an appropriated field-gradient-based magnetic separator. Scanning and transmission electron microscopies were used to investigate the interaction of *Saccharomyces cerevisiae* cells with magnetite nanoparticles. The high-resolution microscopy data suggested that particle incorporation occurs via an active process. Further, the microscopy data shows that the particles did not reach the cell cytoplasm, staying in the periplasmatic space.**

***Index Terms*—Magnetic fluid (MF), scanning electron microscopy (SEM), transmission electron microscopy (TEM), yeast cells.**

I. Introduction

THE use of magnetic materials to support the development of field-gradient magnetic separation technologies has attracted a great deal of attention in recent years [1]–[3]. Applications of such technology span from cell separation [1] to removal of actinides from wastewater [2]. Field-gradient magnetic separation (FGMS) technologies commonly use micron- and nano-sized magnetic particles, which are coupled to the target species for latter removal using a field-gradient-based magnetic device [3]. Magnetic fluids (MFs), once properly engineered to couple to a target, are excellent candidates to support FGMS cleanup technologies addressed to wastewater from the industry. Wastewater containing processed textile dyes, for instance, is an increasing source of environmental contamination. Studies on biodegradation, environmental impact, and health effects of colorant materials, however, reveal the complexity of the subject, not only due to the structural variety of these compounds, but also as a result of the complex composition of effluents which they contaminate. Therefore, the experimental research addressed to the remediation of the environmental effects has been based on several possible strategies. One of them assesses the elucidation of the degradation processes of a limited number of dyes by selected microorganisms [4]. Various strains of yeasts are among the microorganizms used to promote removal and degradation of dyes from wastewater [5]. In addition, very recent data showed that yeast cells (*Saccharomyces cerevisiae*) efficiently interact with magnetic nanoparticles stabilized as low-pH ionic magnetic fluid, leading to the formation of magnetically labeled cells which could be easily separated from the system using an appropriated magnetic separator [6]. However, in order to take full advantage of the formed cell-nanoparticle complex in establishing new technologies one needs to investigate more precisely the nanoparticle cell-site distribution. In this paper, scanning and transmission electron microscopies were used to investigate the interaction of *Saccharomyces cerevisiae* cells with magnetic nanoparticles after incubation of the cells with a low-pH ionic MF sample.

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II. Materials and Methods

Baker's yeast (*Saccharomyces cerevisiae* cells) was incubated with low-pH (perchloric acid stabilization) ionic magnetic fluid. The magnetite-based ionic MF sample was prepared using the standard procedure [7]. The nanoparticle concentration within the MF sample (32.0 mg/mL) is given as the magnetite content determined by a colorimetric method [8]. Procedure used to label the yeast cells with magnetic nanoparticles is described as follows.

Compressed baker's yeast (2 g) was suspended in saline (6 mL), centrifuged, and resuspended in a 6-mL 0.1-M acetate buffer (pH 4.6). After further centrifugation, the sediment was resuspended in acetate buffer to obtain ca 33% yeast suspension (v/v; yeast cells volume determined after sedimentation for 24 h at 1 g). Magnetic labeling of the yeast cells was performed using 3 mL of the yeast suspension and 1 mL of the MF sample. The yeast suspension was mixed with the MF and then incubated at room temperature for one hour without mixing. After this time period the majority of yeast cells were magnetically modified by the added MF sample (the cells responded to external magnetic field). Nonmagnetic yeast cells and residual magnetic fluid were removed by repeated static magnetic separation using acetate buffer (once) and saline as washing liquids, respectively, until the supernatant was clear.

Alternatively, cultured yeast cells were used for magnetic modification. Baker's yeast was suspended in 1% unbuffered saccharose solution and cultivated at 30 °C for 2 h. The cultured cells were centrifuged and treated essentially in

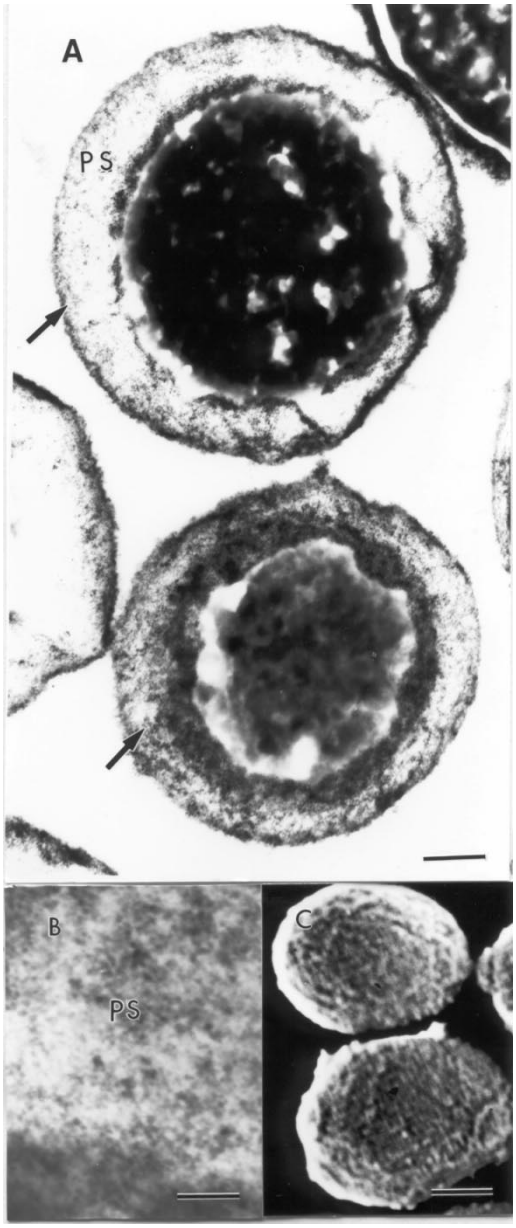

Fig. 1. TEM pictures of G1-cells are shown in A and B. In A (arrows) and B, note the presence of magnetic nanoparticles inside the PS. SEM micrograph of G1-cells is shown in C. Note the bar sizes in A (0.4 μm), B (80 nm), and C (1 μm).

the same way as compressed baker's yeast. Besides labeling with MFs, heating of the yeast cells in boiling water for 15 min was performed as well. The following treatment groups were considered: group G1—cells were first cultured and then incubated with MF; group G2—cells were first cultured, then incubated with MF, and finally heated; group G3—noncultured cells were incubated with MF; group G4—noncultured cells were first incubated with MF and then heated; group G5—cells were first cultured, then heated, and finally incubated with MF; and group G6—noncultured cells were first heated and then incubated with MF. After treatment, cells were harvested, washed, fixed, and processed for scanning electron microscopy (SEM) analysis and transmission electron microscopy (TEM) analysis.

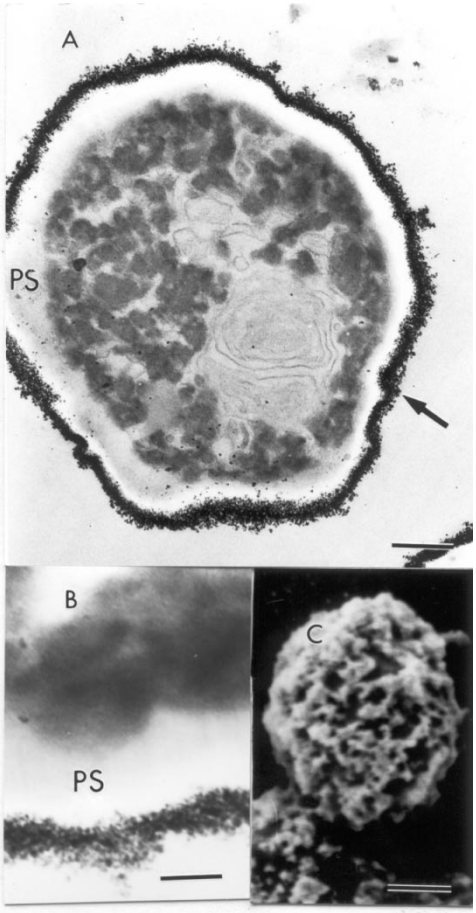

Fig. 2. TEM pictures of G6-cells are shown in A and B. Note in A (arrow) and B, that magnetic nanoparticles are outside the cells instead of in the periplasmatic space (PS). SEM micrograph of G6-cells is shown in C. Very rough surface is observed in C due to agglomeration of magnetic nanoparticles (quite different from C in Fig. 1). Note the bar sizes in A (0.3 μm), B (80 nm), and C (0.7 μm).

III. Results and Discussion

In Fig. 1, SEM micrograph (C) showed smooth cell surface after G1 treatment. G1 and G2 treatments revealed similar results. For these two groups, TEM pictures revealed small number of magnetic nanoparticles on the cell surface and a huge amount of magnetic nanoparticles inside the cells, as showed in Fig. 1 (A and B). Whereas the majority of nanoparticles were observed between the cellular wall and plasmatic membrane, i.e., periplasmatic space (PS), few magnetic nanoparticles were found within the cytoplasm.

In Fig. 2, SEM micrograph (C) showed rough cell surface after G6 treatment, due to agglomerates of magnetic nanoparticles. G3–G6 treatments revealed similar results. In addition, after G3–G6 treatments, the TEM pictures showed very few magnetic nanoparticles inside the cells and a large amount of them on the cell surface, as shown in Fig. 2 (A and B). Regarding groups G3 and G4, where cells were not cultivated and no particles were observed inside the cells, it is clear that the cultivation step is fundamental for MF internalization by the baker's yeast. This result is probably related to the dormant process occurring in the commercial baker's yeast before incubation. After incubation, the cells go into exponential phase of growth, accelerating

TABLE I
G1–G6 CELL TREATMENT

Group	Cell treatment		
G1	cultured	MF-incubated	Heated
G2	cultured	MF-incubated	———
G3	MF-incubated	———	———
G4	MF-incubated	Heated	———
G5	cultured	heated	MF-incubated
G6	heated	MF-incubated	———

their cellular functions, including endocytosis capacity, which is the potential phenomenon behind the incorporation of magnetic nanoparticles by the yeast cells in our experiment. This hypothesis seems to be quite reasonable once groups heated before incubation with MF (G5 and G6) did not internalize magnetic particles. This is a strong indication that nanoparticle internalization is an active instead of a passive phenomenon. Table I summarizes the G1–G6 group treatments.

At this point, it is interesting to observe that cells first cultivated and then treated with MF, before heating (groups G1 and G2), were able to incorporate nanoparticles in the periplasmatic space. Note that magnetic nanoparticles were able to cross the cellular wall, but not the cellular membrane (Fig. 1A and B). It is well known that cellular membrane is more selective than cellular wall, thus supporting our findings. This observation does not interfere with the main goal of the present study, i.e., labeling of yeast cells with magnetic nanoparticles. In addition, neither incubation with MF nor heating after incubation interfere in the baker's yeast capacity in adsorbing dyes. In fact, it has been reported that the dye adsorption capacity is increased when yeast cells are heated for 15 min [6]. Therefore, the order of heating and magnetic modification of yeast cells gain importance from the point of view of cell adsorption capacity.

IV. CONCLUSION

In conclusion, baker's yeast incorporation of magnetic nanoparticles, stabilized as ionic magnetic fluids, is an active process that needs to be performed with cultivated *Saccharomyces cerevisiae* cells. We have observed a significant nanoparticle cell uptake which does not interfere with the dye adsorption capacity of the yeast cells. Therefore, the approach presented in this paper to magnetically load yeast cells represents a way to produce a promising material for environmental bioremediation technologies.

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