Ferrofluid modified *Saccharomyces cerevisiae* cells for biocatalysis

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**Abstract**

An inexpensive and non-toxic magnetically responsive biocatalyst was prepared by ferrofluid modification of *Saccharomyces cerevisiae* cells. The prepared biocatalyst enabled efficient decomposition of hydrogen peroxide (HP) and sucrose conversion, due to the presence of active intracellular catalase and invertase. HP was efficiently decomposed in water solutions up to 2% concentration, leaving very low residual HP concentration after treatment. The biocatalyst was stable; the same catalytic activity was observed after one month storage at 4 °C.

**Keywords:** Magnetic modification, Magnetic fluids, *Saccharomyces cerevisiae*, Hydrogen peroxide

**1. Introduction**

Microbial cells have found numerous applications in different areas of biosciences, biotechnology, chemistry and environmental technology. Many important microorganisms can be easily cultivated and biomass can be obtained in large amounts. Large quantities of waste and dead biomass are also available as by-products of pharmaceutical and enzyme manufacture or the fermentation industry. Microbial cells contain large amount of enzymes which have important applications in food technology and biotechnology. The use of isolated enzymes is advantageous because undesirable by-product formation mediated by contaminating enzymes is minimized. However, isolation and purification of enzymes is in many cases expensive and purified enzymes are frequently less stable under biocatalytic conditions. That is why in many biotransformation processes biocatalysts are used in the form of whole cells (Kafarski & Lejczak, 2004).

Microbial cells can be used both in the free and immobilized form for food technology and biotechnology applications. In general, immobilization or entrapment of microbial cells is usually used to form whole cells biocatalysts with substantially improved possibility of their manipulation. However, manipulation with immobilized cells may be difficult if the separation process is performed in difficult-to-handle samples, such as in particles containing media. In this case magnetic separation techniques can be used with high efficiency. In fact, magnetic carriers and adsorbents have recently showed their importance in many applications. Due to the ferro- or ferrimagnetic properties of the particles and diamagnetic properties of the accompanying molecules and particulate matter, it is possible to separate loaded magnetic adsorbents and carriers even from suspensions with the use of external magnetic field (Safarik & Safariková, 1999; Safarik & Safariková, 2002; Safariková & Safarik, 2001).

Magnetic modification of microbial cells can be performed in different ways including binding of magnetic nano- and microparticles or paramagnetic cations on the cell surface, covalent immobilization on magnetic carriers, entrapment of cells (together with magnetic particles) into biocompatible polymers, cross-linking of cells in the presence of magnetic particles, or by biologically driven precipitation of paramagnetic compounds on the cell surface etc. (Safariková & Safarik, 2007). Recently, magnetic modifications of yeast and algae cells were performed with the use of water based magnetic fluids (ferrofluids). In the simplest way, perchloric acid stabilized magnetic fluid was mixed with cells suspension. After a short period of time magnetic particles precipitated on the cell surface. The prepared magnetically responsive biocomposites were successfully used for the efficient adsorption of water-soluble organic dyes and mercury ions (Safarik et al., 2007; Safariková, Pona, Mosiniewicz-Szablowska, Weyda, & Safarik, 2008; Safarikova, Ptackova, Kibrikova, & Safarik, 2005; Yavuz, Denizli, Gungun, Safarikova, & Safarik, 2006).

The ferrofluid modified microbial cells have been recently usually used in their dead form as magnetic adsorbents for organic and inorganic xenobiotics removal. However, the process of ferrofluid modification may be sufficiently gentle to enable the preparation of magnetically responsive microbial cells with preserved intracellular enzyme activities. Such cells could be used as whole cell biocatalysts.
In the present paper, gentle ferrofluids modification of Saccharomyces cerevisiae (baker’s yeast) cells is described. Magnetic cells were used to catalyze hydrogen peroxide decomposition and invert sugar formation from sucrose, using the intracellular enzymes catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) and invertase (β-D-fructofuranosidase, EC 3.2.1.26). Readily available baker’s yeast cells are of special interest, due to their broad application in food industry; they are classified as “Generally recognized as safe (GRAS)”. Also iron oxides and hydroxides are used in food industry as inorganic colorants (E172). Thus, magnetically responsive S. cerevisiae cells represent a non-toxic, whole cells biocatalyst for potential applications in food industry and biotechnology processes.

2. Materials and methods

2.1. Yeast cells preparation

Baker’s yeast (Linco HEFE, Passau, Germany) was purchased in a local shop. Yeast cells (0.5 g) were suspended in saline and then harvested by centrifugation (Universal 320, Hettich GmbH & Co. KG, Tuttinglen, Germany) at 2370 g for 5 min. The cell pellet was used for magnetic modification. Catalase-free yeast cells were prepared by boiling the cells in saline (15 min) followed by centrifugation. The sediment was suspended in saline and centrifuged again as described above.

2.2. Preparation of magnetically responsive yeast cells

Cell pellet from the previous step was suspended in 2 mL of 0.1 M glycine–NaOH buffer, pH 10.6 and then 0.3 mL of ferrofluid stabilized with tetramethylammonium hydroxide (pH 13.0, concentration 29.1 mg/mL) was added. Alternatively, cells were modified with perchloric acid stabilized ferrofluid (pH 1.7, concentration 26.0 mg/mL) in 0.1 M acetic acid buffer, pH 4.6 and with citrate ferrofluid (pH 6.4, concentration 33.9 mg/mL) in 0.1 M glycine–HCl buffer, pH 2.2. The ferrofluids prepared by standard procedures (Domingo, Mercadal, Petriz, & De Madariaga, 2001; Massart, 1981) were composed of magnetic iron oxides nanoparticles with diameters 10–20 nm. The relative magnetic fluid concentration was given as the iron(II,III) oxide content determined by a colorimetric method (Kiwada, Sato, Yamada, & Kato, 1986). The suspensions were mixed on a sample mixer (Dynal Biotech Inc., NY, USA) for 1 h. The magnetically responsive yeast cells were washed several times by saline and separated by a flat magnetic separator (Qiagen Inc., Valencia, USA). The prepared magnetically responsive yeast cells were stored in saline at 4 °C. Approximately 2.1 mL of sedimented magnetic yeast cells was prepared in one batch.

2.3. Hydrogen peroxide (HP) degradation by modified yeast cells

The effects of various amounts of yeast cells, time of reaction, H2O2 concentrations and repeated application of magnetically responsive yeast cells were assayed at laboratory temperature. The constant volumes of diluted H2O2 solutions (50 mL) were added to magnetic yeast cells and the mixtures were shaken at 125 rpm (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Each experiment was replicated three times (n = 3).

After reaction magnetically responsive yeast cells were removed from the mixture and the remaining H2O2 was determined by a titrimetric method as described (Cefic, 2003). Briefly, 15 mL of sulfuric acid (490 g/L) was added to a mixture of appropriate amount of sample and water to make a total volume of 75 mL. Potassium permanganate (0.25 mol/L) was added to the solution drop by drop under continuous shaking until a slight pink coloration was obtained, which persisted for 30 s. The HP concentration was expressed in percentage and recalculated into millimolar concentration.

2.4. Sucrose hydrolysis by modified yeast cells

The constant volumes of 20% (w/w) sucrose (15 mL) were added to magnetically responsive yeast cells and the mixture was shaken at 125 rpm (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) for 30 min. The amount of glucose formed during sucrose hydrolysis was assayed spectrophotometrically (722–2000 Spectrophotometer, PioMaway Lab Equipment Co., Ltd., Jiangsu, China), using the enzyme test (Glucose GOD 1500, Pliva-Lachema, Brno, Czech Republic). The enzymatic method is based on the oxidation of glucose by glucoseoxidase to gluconate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacted with 4-aminantipyrine and 3-methylphenol to form a colored product, which was measured at 498 nm.

3. Results and discussion

A simple procedure for the preparation of magnetically responsive whole S. cerevisiae cells biocatalysts by their modification with ionic magnetic fluids (ferrofluids) was developed. Three types of ferrofluids were tested for the cells modification. In the case of citrate ferrofluid modification, the prepared magnetically responsive biocomposite was very fine and its complete magnetic separation took a long time (ca. 30 min). The shorter separation time (several minutes) was achieved by magnetically responsive yeast cells modified with tetratetramethylammonium hydroxide stabilized ferrofluid and with perchloric acid stabilized ferrofluid. The magnetic yeast cells modified with tetratetramethylammonium hydroxide stabilized ferrofluid provided significantly better results for tested intracellular enzymes activities (about 40% higher efficiency for HP decomposition and 20% for sucrose hydrolysis, respectively) compared to ferrofluid stabilized with perchloric acid. Therefore, this type of modification was used in all further experiments. The adsorption of magnetic iron oxide nanoparticles onto yeast cells was fast; majority of nanoparticles was adsorbed within several minutes.

Scanning electron microscopy (JEOL JSM-7401F, JEOL Ltd., Japan) confirmed the presence of both single nanoparticles and aggregates externally attached on the cell walls (Fig. 1). Transmission electron microscopy (JEOLJEM-1010, JEOL Ltd., Japan) pictures of the native and magnetically modified cells are presented in Fig. 2; no nanoparticles were found in the periplasmic space or in cytoplasm, which is in correlation with previous findings (Azevedo et al., 2003).

Magnetically modified yeast cells were used for the degradation of H2O2 and sucrose hydrolysis. The preliminary experiments results were compared with those obtained from reactions of the same amounts of yeast cells without magnetic modification and with cells entrapped into magnetic alginate microbeads (Safarik, Sabatková, & Safarikova, 2008; Safarik, Sabatková, & Safarikova, in press). In the case of sucrose hydrolysis 119 mg of yeast cells (unmodified, ferrofluid modified or entrapped into alginate microbeads) reacted with 20% sucrose for 30 min. With unmodified yeast cells 262 mmol/L glucose was formed, corresponding to 44% sucrose conversion. Yeast cells entrapped into magnetic alginate microbeads formed 216 mmol/L glucose (37% sucrose conversion) (Safarik et al., in press), while ferrofluid modified yeast cells formed 163 mmol/L glucose (28% sucrose conversion). Using 430 mg of ferrofluid modified yeast cells 75% sucrose conversion was obtained under the same reaction conditions while the same amount of yeast cells entrapped in magnetic alginate microbeads
(Safarik et al., in press) caused 95% conversion (Fig. 3). Due to the fact that ferrofluid modified yeast cells exhibited worse results than yeast cells encapsulated in magnetic alginate microbeads, no further experiments were performed.

In the case of HP degradation (120 mg of cells, 1 h incubation, 300 mM (ca. 1%) HP concentration), the best results were achieved using both ferrofluid modified yeast cells and unmodified yeast cells (85–86% HP degradation), followed by yeast cells entrapped into magnetic alginate microbeads (69% HP degradation; (Safarik et al., 2008)).

In the first systematic experiments, the dependence of HP decomposition on the amount of ferrofluid modified yeast cells (up to 431 mg) was tested, using 1 h incubation and 337 mM HP concentration. As shown in Fig. 4 application of ferrofluid modified yeast cells provided higher HP decomposition in the whole concentration range than the application of yeast cells entrapped in magnetic alginate microbeads (Safarik et al., 2008).

Based on the previous experiments, constant amount of ferrofluid modified yeast cells (120 mg) was chosen for the following experiments. The study of time dependence of HP decomposition (initial HP concentration 337 mM) has shown that ca. 86% of HP was decomposed after 1 h. After 3 h incubation 99.4% of HP was decomposed.

Initial concentration of HP in the reaction mixture can influence the ability of modified yeast cells to decompose HP in samples. To study this effect, the initial HP concentration in the reaction mixture ranged from 9 mM to 678 mM. Within this HP concentration range, there was constant degree of HP degradation (86%) after one hour incubation.

Reuse of immobilized biocatalysts is one of the necessary assumptions for their potential applications in food and biotechnology industries. Ferrofluid modified yeast cells could be reused at least 3 times in lower initial H₂O₂ concentrations (33 mM

Fig. 1. SEM micrographs of ferrofluid modified Saccharomyces cerevisiae cells showing attached magnetic nanoparticles and their aggregates on the cell surface (bars: 1 μm).

Fig. 2. TEM micrographs of Saccharomyces cerevisiae cells (bars: 1 μm). Top – native cell, bottom – ferrofluid modified cell with attached magnetic iron oxide nanoparticles on the cell wall.

Fig. 3. Dependence of sucrose hydrolysis (expressed as glucose formation) on the amount of magnetic yeast cells. □ Yeast cells entrapped into magnetic alginate microbeads (Safarik et al., in press) • ferrofluid modified yeast cells.
(0.11%) and lower). When the initial HP concentration was higher than 33 mM (0.11%), the residual HP concentration was two times higher during the second application of the same batch of magnetic cells. On the contrary, magnetic alginate microbeads containing entrapped yeast cells could be reused at least 5 times, giving similar results when HP up to 340 mM (ca. 1.16%) initial concentrations were used (Safarik et al., 2008). Most probably, entrapment of cells into alginate matrix protects the cells against the toxic effect of HP at higher concentrations. Our results correspond with the recent study (Chang, Kim, & Shin, 1997) showing that the viability of yeast cells did not change in HP concentrations up to 40 mM (ca. 0.14%).

The ferrofluid modified yeast cells were stable at 4 °C in saline for at least 1 month. There was very low change in HP decomposition efficiency.

Catalase-free yeast cells and catalase-free ferrofluid modified yeast cells were prepared to check the possible non-enzymatic HP decomposition. It was shown that dead cells have no effect on HP decomposition. Ferrofluid modified catalase-free yeast cells caused ca 1.5% HP decomposition after 1 h incubation at 340 mM HP initiation concentration.

It can be concluded that ferrofluid modified yeast cells could be used for HP decomposition. Their efficiency and stability are high and are comparable with those of native cells. For applications where high HP concentration is expected and for repeated applications, yeast cells entrapped in magnetic alginate microbeads are preferable.

Ferrofluid modified yeast cells exhibited also relatively high invertease activity enabling efficient sucrose conversion but better results were obtained using cells entrapped in magnetic alginate microbeads.

4. Conclusions

Ferrofluid modification of yeast cells performed under the described conditions did not lead to the substantial changes in intra-cellular enzyme activities, as shown on the example of intracellular catalase and invertase (enabling the hydrogen peroxide decomposition and sucrose conversion, resp.). Ferrofluid modified S. cerevisiae cells can thus be used as non-toxic and efficient magnetically responsive whole cell biocatalysts.

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References


