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PMAA-stabilized ferrofluid/chitosan/yeast composite for bioapplications

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ABSTRACT

A simple, one-pot process for the preparation of magnetically responsive yeast-based biocatalysts was developed. *Saccharomyces cerevisiae*, *Candida utilis* and *Kluyveromyces lactis* cells were successfully incorporated into chitosan gel magnetically modified with poly(methacrylic acid)-stabilized magnetic fluid (PMAA-FF) during its formation. Magnetic PMAA-FF/chitosan/yeast composites were efficiently employed for invert sugar production. The dependence of invertase activity on used yeast, amount of magnetic biocatalyst, agitation time and after reuse was studied in detail. The tested magnetic biocatalysts retained at least 69% of their initial activity after 8 reuse cycles.

1. Introduction

Microbial cells can serve as inexpensive substitutes of purified enzymes. In addition to their low cost consisting in no need of isolation and purification of enzymes, the whole-cell biocatalysts can also provide higher stability of enzyme and its repeated use. Therefore, whole yeast, bacterial and algal cells have found many interesting applications in various areas of biosciences [1], food industry [2,3], biotechnology [4,5] and environmental technology [6,7], and have been efficiently used either in free or immobilized form [7].

Despite the existence of many immobilization techniques, microbial cells are often immobilized or entrapped in polymer matrices of different origin. Both natural polymers, represented by alginate [8,9], agar/agarose [10,11] or carrageenan [12,13], and synthetic ones, such as polyacrylamide [14] or polyvinylalcohol [15,16], have recently been employed as carriers for immobilization of diverse microorganisms. Among them, chitosan (a linear polysaccharide composed of randomly distributed β-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine) has also been used several times [17,18]. This polymer provides a unique set of characteristics: biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, antibacterial properties, heavy metal ions chelation, gel forming properties and hydrophilicity, and remarkable affinity to proteins [19]. In the biomedical domain (for which biocompatibility is essential), chitosan is used to prepare hydrogels, films, fibers or sponges [20].

Magnetic derivatives of whole-cell biocatalysts can be prepared to facilitate their manipulation in suspensions and other difficult-to-handle media. Binding of magnetic iron oxide nano- or microparticles in form of magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) or their mixtures enables simple, rapid and selective separation of magnetically labeled biocomposites by means of external magnetic field (permanent magnet, commercially available magnetic separators, electromagnets). Magnetically responsive biocatalysts can be prepared by various 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 [21]. Some reviews on this topic have been written recently [22–24].

Numerous research papers have been focused on simple magnetic modification using magnetic fluid (ferrofluid, FF), a colloidal homogeneous suspension of magnetic iron oxides or ferrite nanoparticles in polar or nonpolar carrier liquids. Iron oxide nanoparticles in magnetic suspension have to be stabilized against aggregation; stabilization is usually carried out by perchloric acid, tetramethylammonium hydroxide or by diverse polymers including dextran or starch. In this work, recently developed and exhaustively characterized poly(methacrylic acid)-stabilized FF (PMAA-FF) [25] has been employed.

Magnetically responsive immobilized yeast cells have been used, for

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instance, as whole-cell biocatalysts utilized for ethanol production [26,27], hydrogen peroxide removal [28] and sucrose hydrolysis [2,29], or as efficient adsorbents of various xenobiotics, such as organic water-soluble dyes [30,31], heavy metal ions [32–34] or radionuclides [35,36].

The main aim of this study is a comparison of invertase activity of three magnetically responsive chitosan/yeast biocatalysts under various experimental conditions used.

2. Materials and methods

2.1. Materials

Poly(methacrylic acid) sodium salt (PMAA, 30 wt% solution in water, M_w=9 500 g/mol), $FeCl_3 \cdot 6H_2O$, and $FeCl_2 \cdot 4H_2O$ were from Sigma-Aldrich (USA). Chitosan (medium molecular weight ca 400,000, 75–85% deacetylated) was supplied by Fluka (Switzerland), Glukosa GOD 1500 kit by Pliva-Lachema (Czech Republic) and acetic acid by Lachema (Czech Republic). NaCl, HCl, 25% NH_3 in H_2O, sucrose and D-glucose were obtained from Lachner (Czech Republic). All chemicals were of GR grade. Malt Extract Broth medium was supplied by Oxoid (United Kingdom). *Saccharomyces cerevisiae* cells (baker´s yeast) were purchased in a local supermarket, *Candida utilis* and *Kluyveromyces lactis* were from microbiological collection of Department of Nanobiotechnology.

2.2. Cell cultivation

Compressed *S. cerevisiae* cells were suspended is saline. *C. utilis* and *K. lactis* were cultivated in 500 mL of Malt Extract Broth for 3 days at 28 °C under mild shaking (90 rpm, shaker Heidolph, Germany). To dispose cultivation medium, cells were repeatedly centrifuged (Zentrifugen Universal 320, Hettich, Germany) at 442 (*C. utilis*) and 996 (*K. lactis*) *g* for 5 min and washed with saline. The washed cell suspension was allowed to settle in 50 mL calibrated cylinders under earth gravity at 4 °C for 18 h.

2.3. Preparation of PMAA-FF

PMAA-FF was prepared as described previously [25]. Briefly, PMAA solution (25 mL) was mixed with 50 mL of 2 M HCl. Ferric chloride (5.0 *g*) and ferrous chloride (2.1 *g*) were dissolved together in 13 mL of hydrochloric acid (2 mol/L). Both solutions were mixed together (465 rpm, Heidolph RZR 2041, Germany) and heated (on a water bath) to 60 °C. Then 75 mL of 7.5% ammonium hydroxide solution was added dropwise under mixing. The formed ferrofluid was then incubated at 60 °C for the next 15 min. Free ammonium gas was allowed to evaporate at room temperature (RT) in a fume hood for 24 h and then the ferrofluid was centrifuged at 2767 g for 45 min (Hettich Universal 320, Germany). In order to increase concentration of magnetic iron oxide particles in the ferrofluid, water was evaporated at room temperature in a fume hood for several days.

2.4. Preparation of PMAA-FF/chitosan/yeast composite

0.1 *g* of chitosan was dissolved in 20 mL of 0.1 M acetic acid. After dissolving, the volume was made up to 200 mL with saline and 50 mL of cell suspension (5 mL of settled volume of yeast in saline) was added under stirring at 400 rpm (Head Stirrer Heidolph, Germany). Then, 3 mL of PMAA-FF (c=72.5 mg/mL, pH 6.3) was slowly added and the mixing continued for another 10 min. Prepared magnetically responsive biocatalyst was repeatedly magnetically separated and washed with saline to remove the free magnetic particles and unbound cells. Thoroughly washed magnetic PMAA-FF/chitosan/yeast composite was allowed to settle in 25 mL calibrated cylinders under earth gravity at 4 °C for 18 h. One batch of each biocatalyst was prepared.

2.5. Characterization of PMAA-FF/chitosan/yeast composite

The morphological study was carried out using Scanning Electron Microscopy (SEM) measurements; the samples were analyzed by 120 Hitachi SU6600 scanning electron microscope (Hitachi, Japan) with accelerating voltage 1 kV, equipped with Energy Dispersive Spectroscopy (EDS) – Thermo Noran System 7 (Thermo Scientific, MA, USA) with Si(Li) detector (accelerating voltage of 10 kV and acquisition time 300 s).

2.6. Enzyme assay

Dependence of invertase activity on the type of used yeast cells, amount of biocatalyst, agitation time and after reuse was tested. Amount of glucose formed was determined using commercially available kit. In the case of time study, 300 μL of settled volume of biocatalyst was incubated for 5–150 min. For determination of activity dependence on biocatalyst amount, 50–400 μL of settled cells were shaken for 30 min. The invertase activity after reuse was evaluated using 300 μL of settled cells incubated for 30 min; 8 reuse cycles were examined (biocatalyst was 3 times magnetic separated and washed with saline before reuse).

Cell suspension was shaken at 150 rpm in 50 mL beakers containing 15 mL of 20% sucrose. After incubation of cells in sucrose, the cells were magnetically separated (using a flat magnet) and the clear supernatant was utilized; 20 μL of diluted sample was added to 2 mL of kit reaction solution, followed by incubation in the dark for 30 min. Absorbance of sample at 498 nm was measured using spectrophotometer (Cintra 20, GBC Scientific Equipment, Australia). All experiments were carried out in triplicate and each sample was diluted before glucose determination twice; average values are presented.

3. Results and discussion

S. cerevisiae, C. utilis and *K. lactis* cells were successfully incorporated into chitosan treated with PMAA-stabilized ferrofluid during its formation. This simple and fast process led to the formation of magnetically responsive biocatalyst that could be easily separated using external magnetic field, as demonstrated in Fig. 1. Prepared magnetic composite containing *S. cerevisiae* cells can be stored for one month at 4 °C without loss of its magnetic properties or enzyme activity [25]. Similar results can also be expected in the case of *C. utilis* and *K. lactis* biocatalysts where the enzyme activity remained unaltered after 14 days of storage (data not shown).

PMAA-FF and PMAA-FF/chitosan particles were characterized in

Fig. 1. Magnetic separation of PMAA-FF/chitosan/*K. lactis* biocomposite.

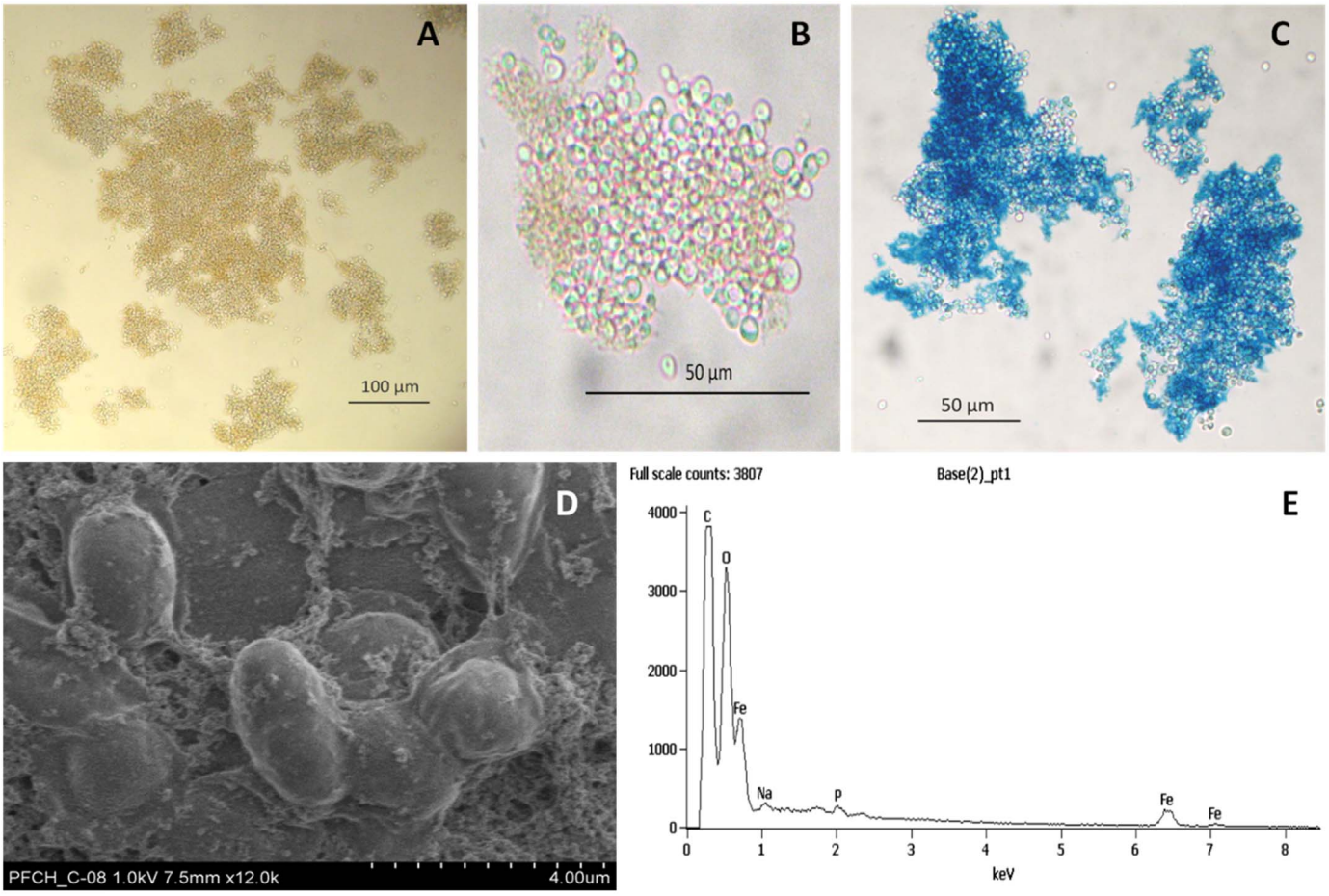

Fig. 2. (A–C) Appearance of PMAA-FF/chitosan/*K. lactis* composite; A) flocs of different sizes; B) detail image of immobilized cells, C) composite after Perls' Prussian blue staining. (D–E) Characterization of PMAA-FF/chitosan/*C. utilis* composite; D) SEM image, E) EDS spectrum. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

detail previously [25] by means of a combination of microscopy (optical microscopy, transmission and scanning electron microscopy), scattering (static and dynamic light scattering, small-angle neutron scattering) and spectroscopy (Fourier transform infrared spectroscopy) techniques. The presence of nanoparticles of magnetite/maghemite crystalline structure forming agglomerates with typical size about 100 nm was proved.

Morphology of PMAA-FF/chitosan/yeast composites studied by optical microscopy revealed the brown-reddish flocs of chitosan in irregular shapes and sizes containing immobilized yeast cells (Fig. 2A, B). The presence of magnetic particles was successfully confirmed by Perls' Prussian Blue Staining causing intensive blue coloration of chitosan (Fig. 2C), by SEM and EDS (Fig. 2D, E).

Invert sugar formation (hydrolysis of sucrose into its basic building components – glucose and fructose – in ratio 1:1) is enabled by the enzyme invertase, also called β-D-fructofuranosidase (E.C. 3.2.1.26). In the food industry, enzyme obtained from *S. cerevisiae* cells is primarily employed for this purpose. However, other yeast species can also exhibit invertase activity, although with slightly lowered efficiency.

Using the same experimental conditions (0.3 mL of settled cells, 120 min incubation in 15 mL of 20% sucrose, at RT), the new magnetic biocatalysts consisted of *C. utilis* and *K. lactis* cells produced 364 ± 9 and 219 ± 5 mM of glucose (Fig. 3), while the recently developed PMAA-FF/chitosan composite containing entrapped *S. cerevisiae* cells formed 468 ± 23 mM of glucose [25]. Despite the lower values of produced invert sugar compared with commonly used yeast, both tested composites (especially the one with *C. utilis* cells) can be still considered to be sufficient biocatalysts applicable as an alternative to *S. cerevisiae* cells.

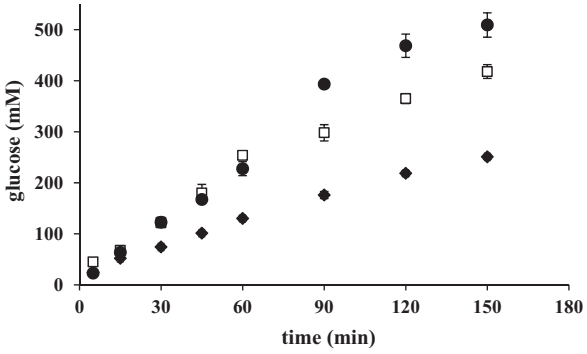

Fig. 3. Time course of sucrose hydrolysis catalyzed by □ *C. utilis*, • *S. cerevisiae* and ♦ *K. lactis* (0.3 mL of settled volume, RT).

Dworschack and Wickerham [37] extensively studied the production of extracellular and total invertase in 68 species in 17 genera of yeast, and concluded that the invertase activity of *S. cerevisiae* is inferior to *C. utilis* under experimental conditions used (1 h shaking at 20 °C). To compare with magnetic yeast-based biocatalysts presented in this study (Fig. 3), the composite containing *C. utilis* also exhibited slightly higher invert sugar production than the one with *S. cerevisiae* cells after 60 min of shaking. Nevertheless, when incubation time longer than 90 min was used, the efficiency in invert sugar production became opposite.

The invertase activity was dependent not only on used cells or

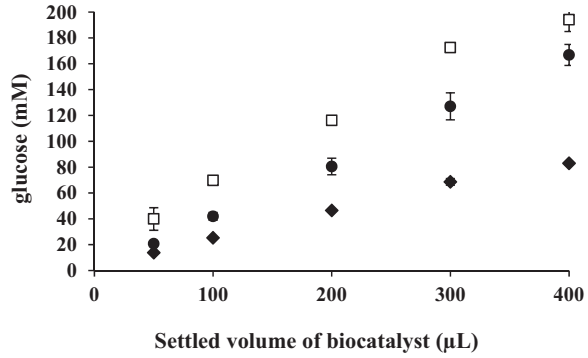

Fig. 4. Dependence of amount of biocatalyst (□ *C. utilis*, • *S. cerevisiae* and ◆ *K. lactis)* on invert sugar formation (30 min incubation, RT).

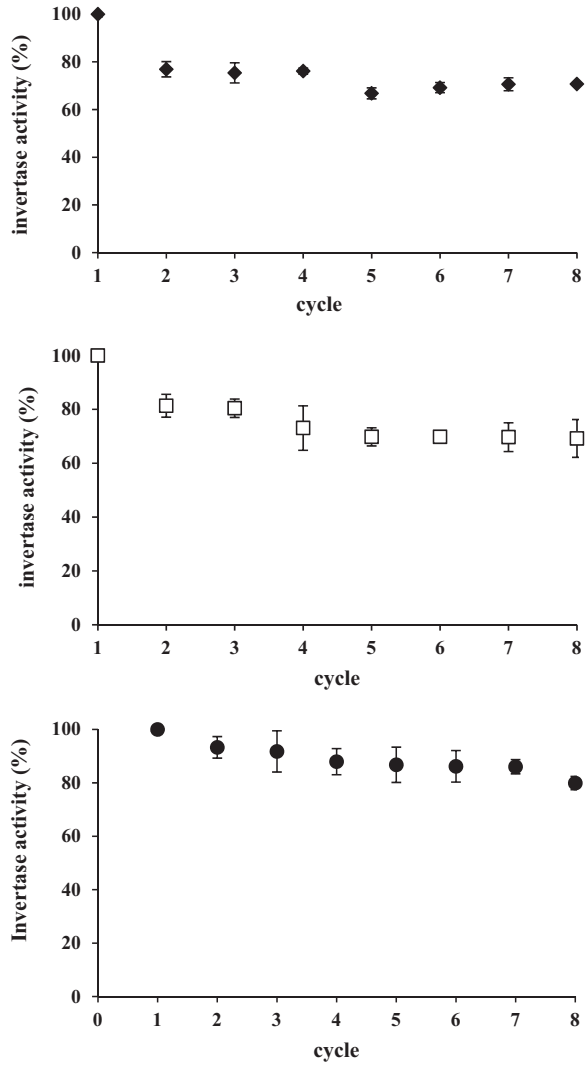

Fig. 5. Invertase activity of PMAA-FF/chitosan/yeast composite after reuse (0.3 mL settled cells: ◆ *K. lactis*, □ *C. utilis*, • *S. cerevisiae*; RT, 30 min incubation).

incubation time, but also on the amount of yeast cells in reaction mixture. As can be seen from Fig. 4, 100 µL of settled PMAA-FF/chitosan/*C. utilis* composite produced 73 ± 2 mM of glucose (after 30 min of incubation), while almost 2.5x higher value (179 ± 5 mM) was obtained using 300 µL of tested biocatalyst. The same trend can be observed in the case of *K. lactis* and *S. cerevisiae* composite.

The reuse of immobilized catalysts is a significant parameter for their potential application in food and biotechnology industry; therefore, the invertase activity after 8 cycles was studied. Fig. 5 demonstrates that *K. lactis* composite retained 76% of its initial activity after 4 cycles. Then a slight decrease at 69–70% is apparent. *C. utilis* exhibited ca 80% of initial activity during three cycles. After the 4th cycle, the activity dropped at 73% and then remained constant at 69%. The highest invertase activity after reuse was observed for *S. cerevisiae* composite. In this case, a gradual fall to 79% during the 8 cycles can be observed. Obtained results clearly indicated that all tested biocomposite could be repeatedly used with relatively high efficiencies retained.

4. Conclusions

New biocatalysts containing *K. lactis* and *C. utilis* cells entrapped in biocompatible chitosan gel magnetically labeled with recently developed poly(methacrylic acid)-stabilized ferrofluid were prepared by a fast, simple and one-pot technique. Invertase activity of both biocatalysts was compared with the same matrix containing *S. cerevisiae* cells usually employed for industrial invert sugar production. The incubation time is the most significant factor for determination of efficiency of yeast-based biocatalyst. When used for less than 60 min, the highest invert sugar yield was observed for composite containing *C. utilis* cells. Nevertheless, after 90 min of shaking in sucrose solution, the amount of produced invert sugar using *S. cerevisiae* composite was higher than in the case of *C. utilis* biocatalyst. Based on these results, the invertase activity of tested yeast-based biocatalysts after 90 min is as follows: *S. cerevisiae* > *C. utilis* > *K. lactis*. All magnetically responsive biocatalysts were stable and can be used for 8 cycles with maximum of 31% loss of their initial activity.

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References

[1] S.U. Kadam, B.K. Tiwari, C.P. O'Donnell, Application of novel extraction technologies for bioactives from marine algae, J. Agric. Food Chem. 61 (2013) 4667–4675.
[2] I. Safarik, Z. Sabatkova, M. Safarikova, Invert sugar formation with *Saccharomyces cerevisiae* cells encapsulated in magnetically responsive alginate microparticles, J. Magn. Magn. Mater. 321 (2009) 1478–1481.
[3] F. Bourdichon, S. Casaregola, C. Farrokh, J.C. Frisvad, M.L. Gerds, W.P. Hammes, J. Harnett, G. Huys, S. Laulund, A. Ouwehand, I.B. Powell, J.B. Prajapati, Y. Seto, E. Ter Schure, A. Van Boven, V. Vankerckhoven, A. Zgoda, S. Tuijtelaars, E.B. Hansen, Food fermentations: microorganisms with technological beneficial use, Int. J. Food Microbiol. 154 (2012) 87–97.
[4] A. Anjum, M. Zuber, K.M. Zia, A. Noreen, M.N. Anjum, S. Tabasum, Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: a review of recent advancements, Int. J. Biol. Macromol. 89 (2016) 161–174.
[5] J.R. Miranda, P.C. Passarinho, L. Gouveia, Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production, Bioresour. Technol. 104 (2012) 342–348.
[6] R. Khan, P. Bhawana, M.H. Fulekar, Microbial decolorization and degradation of synthetic dyes: a review, Rev. Environ. Sci. Bio-Technol. 12 (2013) 75–97.
[7] S. Srivastava, S.B. Agrawal, M.K. Mondal, A review on progress of heavy metal removal using adsorbents of microbial and plant origin, Environ. Sci. Pollut. Res. 22 (2015) 15386–15415.
[8] S.K. Bhatia, Y.H. Kim, H.J. Kim, H.M. Seo, J.H. Kim, H.S. Song, G. Sathiyanarayanan, S.H. Park, K. Park, Y.H. Yang, Biotransformation of lysine into cadaverine using barium alginate-immobilized *Escherichia coli* overexpressing CadA, Bioprocess Biosyst. Eng. 38 (2015) 2315–2322.
[9] S. Behera, R.C. Ray, Batch ethanol production from cassava (*Manihot esculenta* Crantz.) flour using *Saccharomyces cerevisiae* cells immobilized in calcium alginate, Ann. Microbiol. 65 (2015) 779–783.
[10] A.K. Dolui, S. Das, Comparative study of 6-APA production by free and agar immobilized bacteria in nutrient broth culture, Indian J. Exp. Biol. 49 (2011) 289–292.
[11] T. Takei, T. Kamagasako, Y. Yuzi, N. Tomioka, M. Yoshida, Comparison of *Rhodococcus erythropolis* CS98 strain immobilized in agarose gel and PVA gels for accumulation of radioactive Cs-137, J. Chem. Eng. Jpn. 48 (2015) 782–786.
[12] A.K. Dolui, S. Sahana, A. Kumar, Studies on production of 6-aminopenicillanic acid by free and kappa-carrageenan immobilized soil bacteria, Indian J. Pharm. Educ.

Res. 46 (2012) 70–74.
[13] S.K.P. Kumar, V.H. Mulimani, Immobilization of *Aspergillus oryzae* with kappa-carrageenan for soybean oligosaccharide hydrolysis, Food Sci. Biotechnol. 20 (2011) 1691–1697.
[14] M. Awais, A. Pervez, A. Yaqub, M.M. Shah, Production of antimicrobial metabolites by *Bacillus subtilis* immobilized in polyacrylamide gel, Pak. J. Zoology 42 (2010) 267–275.
[15] M.H. El-Naas, A.H.I. Mourad, R. Surkatti, Evaluation of the characteristics of polyvinyl alcohol (PVA) as matrices for the immobilization of *Pseudomonas putida*, Int. Biodeterior. Biodegrad. 85 (2013) 413–420.
[16] M. Rebros, M. Rosenberg, R. Stloukal, Immobilization of cells and enzymes to PVA gel, New Biotech. 31 (2014) (S89-S89).
[17] I. Safarik, K. Pospiskova, Z. Maderova, E. Baldikova, K. Horska, M. Safarikova, Microwave-synthesized magnetic chitosan microparticles for the immobilization of yeast cells, Yeast 32 (2015) 239–243.
[18] Z. Yang, Immobilization of *Enterococcus faecalis* cells with chitosan: a new process for the industrial production of L-citrulline, Process Biochem. 50 (2015) 1056–1060.
[19] B. Krajewska, Application of chitin- and chitosan-based materials for enzyme immobilizations: a review, Enzym. Microb. Technol. 35 (2004) 126–139.
[20] M. Rinaudo, Chitin and chitosan: properties and applications, Prog. Polym. Sci. 31 (2006) 603–632.
[21] I. Safarik, M. Safarikova, Magnetically modified microbial cells: a new type of magnetic adsorbents, China Part. 5 (2007) 19–25.
[22] I. Safarik, Z. Maderova, K. Pospiskova, E. Baldikova, K. Horska, M. Safarikova, Magnetically responsive yeast cells: methods of preparation and applications, Yeast 32 (2015) 227–237.
[23] I. Safarik, K. Pospiskova, E. Baldikova, Z. Maderova, M. Safarikova, Magnetic modification of cells, in: A. Grumezescu (Ed.)Engineering of NanoBioMaterials: Applications of NanoBioMaterials, Elsevier, US, 2016, pp. 145–181.
[24] I. Safarik, Z. Maderova, K. Pospiskova, K. Horska, M. Safarikova, Magnetic decoration and labeling of prokaryotic and eukaryotic cells, in: R. Fakhrullin, I. Choi, Y. Lvov (Eds.), Cell Surface Engineering: Fabrication of Functional Nanoshells, The Royal Society of Chemistry, 2014, pp. 185–215.
[25] I. Safarik, M. Stepanek, M. Uchman, M. Slouf, E. Baldikova, L. Nydlova, K. Pospiskova, M. Safarikova, Composite particles formed by complexation of poly(methacrylic acid) – stabilized magnetic fluid with chitosan: magnetic material for bioapplications, Mater. Sci. Eng. C 67 (2016) 486–492.
[26] C.Z. Liu, F. Wang, F. Ou-Yang, Ethanol fermentation in a magnetically fluidized bed reactor with immobilized *Saccharomyces cerevisiae* in magnetic particles, Bioresour. Technol. 100 (2009) 878–882.
[27] V. Ivanova, P. Petrova, J. Hristov, Application in the ethanol fermentation of immobilized yeast cells in matrix of alginate/magnetic nanoparticles, on chitosan-magnetite microparticles and cellulose-coated magnetic nanoparticles, Int. Rev. Chem. Eng. 3 (2011) 289–299.
[28] I. Safarik, Z. Sabatkova, M. Safarikova, Hydrogen peroxide removal with magnetically responsive *Saccharomyces cerevisiae* cells, J. Agric. Food Chem. 56 (2008) 7925–7928.
[29] K. Pospiskova, G. Prochazkova, I. Safarik, One-step magnetic modification of yeast cells by microwave-synthesized iron oxide microparticles, Lett. Appl. Microbiol. 56 (2013) 456–461.
[30] Q. Wu, Z. Shan, M. Shen, S. Li, H. Chen, Biosorption of direct scarlet dye on magnetically modified *Saccharomyces cerevisiae* cells, Chin. J. Biotechnol. 25 (2009) 1477–1482.
[31] J.X. Yu, L.Y. Wang, R.A. Chi, Y.F. Zhang, Z.G. Xu, J. Guo, A simple method to prepare magnetic modified beer yeast and its application for cationic dye adsorption, Environ. Sci. Pollut. Res. 20 (2013) 543–551.
[32] M. Xu, Y.S. Zhang, Z.M. Zhang, Y. Shen, M.J. Zhao, G.T. Pan, Study on the adsorption of Ca^{2+}, Cd^{2+} and Pb^{2+} by magnetic Fe_3O_4 yeast treated with EDTA dianhydride, Chem. Eng. J. 168 (2011) 737–745.
[33] L. Uzun, N. Saglam, M. Safarikova, I. Safarik, A. Denizli, Copper biosorption on magnetically modified yeast cells under magnetic field, Sep. Sci. Technol. 46 (2011) 1045–1051.
[34] Y. Zhang, J. Zhu, L. Zhang, Z. Zhang, M. Xu, M. Zhao, Synthesis of EDTAD-modified magnetic baker's yeast biomass for Pb^{2+} and Cd^{2+} adsorption, Desalination 278 (2011) 42–49.
[35] J. Bai, X. Wu, F. Fan, W. Tian, X. Yin, L. Zhao, F. Fan, Z. Li, L. Tian, Z. Qin, J. Guo, Biosorption of uranium by magnetically modified *Rhodotorula glutinis*, Enzym. Microb. Technol. 51 (2012) 382–387.
[36] Y.Q. Ji, Y.T. Hu, Q. Tian, X.Z. Shao, J. Li, M. Safarikova, I. Safarik, Biosorption of strontium ions by magnetically modified yeast cells, Sep. Sci. Technol. 45 (2010) 1499–1504.
[37] R.G. Dworschack, L.J. Wickerham, Production of extracellular and total invertase by *Candida utilis*, *Saccharomyces cerevisiae*, and other yeasts, Appl. Microbiol. 9 (1961) 291–294.