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Immobilization of Glucose Oxidase on Synthesized Superparamagnetic Fe_3O_4 Nanoparticles; Application for Water Deoxygenation

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Abstract— Enzymatic removal of dissolved oxygen by using glucose-glucose oxidase immobilized on magnetic nanoparticles of Fe_3O_4 system was performed. Magnetic nanoparticles of Fe_3O_4 were prepared via improved chemical co-precipitation method. Fourier Transform Infrared Spectroscopy (FT-IR), X-ray diffraction (XRD), transmission electron microscopy (TEM), Brunauer–Emmett–Teller (BET), and vibrating sample magnetometer (VSM) analyses confirmed the formation of these nanoparticles. The crystal sizes of the magnetic nanoparticles of Fe_3O_4 were calculated approximately 19 nm via XRD by Williamson-Hall equations. The saturation magnetization value of Fe_3O_4 nanoparticles was obtained 68.8 emu/g. Fe_3O_4 nanoparticles specific surface area was obtained 62.7m^2/g that it is appropriate for enzyme immobilization. Glucose oxidase immobilization was done by physical method; and percentage of immobilization was obtained 78% with specific activity of 640U/g. Deoxygenation of 150 ml of tap water with 6.9 mg/L O_2 which was catalyzed by the 0.15g immobilized glucose oxidase in the presence of twice stochiometric amount of glucose was completed in about four min and 20 s at 35ºC.

Index Terms— Dissolved oxygen, Deoxygenation, Glucose oxidase, Fe_3O_4, Magnetic nanoparticles.

1 INTRODUCTION

THE unique characterizations of super paramagnetic materials propose them in the very wide range of applications [1-4]. In recent years, magnetic nanoparticles have been used in information storage [5], color imaging [6], magnetic refrigeration [7], bioprocessing and bionanotechnology [8], medical diagnosis [9], controlled drug delivery [10] and as ferrofluids [11]. Metallic nanoparticles of Co, Ni, and Fe have a higher magnetization than metal oxides, but they are highly reactive and toxic, so, they aren't suitable for some cases such as enzyme immobilization and biomedical applications [10]. Iron oxide nanoparticles are interested due to their oxidative stability, compatibility in nonaqueous systems, nontoxicity, and appropriate groups on the surface for mentioned applications [11-13], for example, as support for enzyme immobilization [14].

Enzymes are used as biocatalysts in the many chemical, pharmaceutical and food industries due to their ability for catalyzing reactions in the suitable procedure [15]. For overcoming the some drawbacks of enzymes such as high-cost and low stability, enzyme immobilization is a suitable way. There are various techniques such as physical adsorption, covalent binding, entrapment, and cross linking to enzyme immobilization [16]. Every technique has some advantages and some drawbacks; Immobilization by physical adsorption is caused by interaction between enzyme and support such as electrostatic and hydrophobic interactions or hydrogen bonds. In the last few decades, adsorptive enzyme immobilization techniques have been intensively studied, because of some advantages including high efficiency, reversibility and simplicity, which enable enzyme immobilization under mild conditions [17].

Varieties of supports have been used for immobilization of glucose oxidase (GOx) such as mesoporous MnO_2, Al_2O_3, SiO_2, glassy carbon, Au nanoparticals, stainless steel, anion exchange resin, and etc for different goals [18-25]. Enzyme immobilization on magnetic nanoparticles of iron oxide has some important advantages. When enzymes immobilized on magnetic nanoparticles of iron oxide, can be easily separated from the reaction medium by the use of an external magnetic field where the substrate solution is removed while the immobilized enzyme is held in place or vice versa. This system provides a relatively simple technique for separating and reusing enzymes for a longer period than free enzymes and also immobilized enzymes on nonmagnetic supports [26-28]. So, the use of magnetic supports can reduce the operation costs, and therefore the total cost [29].

The main aim of this research is synthesis and structural study of magnetic nanoparticles of Fe_3O_4 as a support for immobilization of GOx for for water deoxygenation which is suitable support in compare of other supports [29].

In many industries water deoxygenation is a necessary step because dissolved oxygen (DO) causes some problems such as pitting corrosion [30,31]. Methods developed for this means are classified into two categories i.e. physical and chemical methods. Physical methods in turn include thermal degassing, vacuum degassing, nitrogen bubbling and degassing through a membrane module [32-34]. Chemical methods on the other

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hand include use of a reducing agent such as hydrazine, sodium sulfite and hydrogen in different procedures [35-38]. All of them have some defects from different aspects such as low efficiency, toxicity, economical problems and hard working conditions. In this research water deoxygenation through the immobilized GOx on magnetic nanoparticles of iron oxide was performed in a stirred tank reactor.

2 EXPERIMENTAL

2.1 Materials

Glucose oxidase (GOx) type II (EC 1.1.3.4, 50 U/mg, from Aspergillus niger), Bovine liver catalase (EC 1.11.1.6, 3809 U/mg), β-D-(+)-glucose, sodium acetate, acetic acid, 2,2′-Azino-di-[3-ethylbenzthiazolin-sulfonate] (ABTS), $FeCl_2.4H_2O$, $FeCl_3.6H_2O$, $NH_3.H_2O$, and nitrogen gas in the analytical grade were obtained.

2.2 Preparation of the magnetic nanoparticles of Fe_3O_4

Magnetic nanoparticles of Fe_3O_4 were synthesized via improved chemical co-precipitation method. 3.1736g of $FeCl_2.4H_2O$ (0.016 mol) and 7.5684g of $FeCl_3.6H_2O$ (0.028 mol) were dissolved in 320 ml of de-ionized water. The mixed solution was stirred under nitrogen atmosphere at 80°C for 1 h. Then, 40 ml of $NH_3.H_2O$ was injected into the mixture rapidly and stirred under nitrogen gas at 80°C for another 1 h and then cooled to room temperature. The precipitated particles were washed five times with hot water and separated by magnetic decantation. Finally, Fe_3O_4 magnetic nanoparticles were dried under vacuum at 70°C [39].

2.3 Characterization of the synthesized magnetic nanoparticles of Fe_3O_4

Fourier Transform Infrared Spectroscopy (FT-IR) was recorded by a Tensor 27 Model (Bruker German) spectrophotometer using KBr pellets for study functional groups of the synthesized Fe_3O_4 before and after immobilization. The X-ray diffraction (XRD) pattern of the as-prepared sample was determined using a D5000 diffractometer (Siemens German) with Cu Kα radiation source (λ=1.54056Å). Morphology of the sample was examined by transmission electron microscopy (TEM) Leo 1455 VP Model with 10kV voltage. Specific surface area and pore volume of the prepared Fe_3O_4 were obtained through the Brunauer–Emmett–Teller (BET) with micromeritics Gemini 2375 (USA) analyzer. Magnetic behavior of synthesized Fe_3O_4 was studied at room temperature using a Vibrating Sample Magnetometer AGFM before and after immobilization.

2.4 Assay of GOx activity

GOx oxidizes β-D-glucose in the presence of oxygen to β-D-glucono-δ-lactone and H_2O_2. The produced H_2O_2 is then utilized to oxidize a chromogenic substrate in a secondary reaction in the presence of catalase and a resultant color change is monitored spectrophotometrically. 2, 2′- Azino– di- [3 - ethylbenzthiazolin-sulfonate] (ABTS) was used for this goal through forming a greenish-blue oxidized product that was measured spectrophotometrically at 420 nm [40]. One unit of catalyst activity (U) is defined as the amount of GOx required to consume 1μmol substrate in one min at 25°C [40].

2.5 Immobilization of GOx

1 g of the magnetic nanoparticles of Fe_3O_4 was dispersed in 10 ml of 100U/ml GOx solution in acetate buffer at pH 5.50 with shaking in a shakerincubator for 90 min at 20°C. The obtained hybrid catalyst was separated from solution by using an external magnetic field and decantation and then was stored at 4°C until use [29].

2.6 Assay of the GOx- Fe_3O_4 hybrid catalyst activity

Glucose oxidase immobilized in Fe_3O_4 magnetic nanoparticles directly converts glucose to gluconic acid and H_2O as depicted in (Eq. 1). Glucose solution (0.10M) was filled into a precisely sealed mixed reactor. 0.15g GOx-Fe_3O_4 was added to the reactor and deoxygenation reaction was started up. Assay of the hybrid catalyst activity was carried out by monitoring of DO concentration decreasing during one min using DO meter sensor (WinLab Art. 6103 30030 German). One unit of hybrid catalyst activity (U) in this research has been defined as the amount of catalyst required to consume 1μmol O_2 per min at 25°C [29].

$$\beta\text{-D-glucose} + 1/2\ O_2 \xrightarrow{\text{GOx-Fe}_3\text{O}_4} \text{D-gluconic acid} + H_2O \quad (1)$$

3 RESULTS AND DISCUSSION

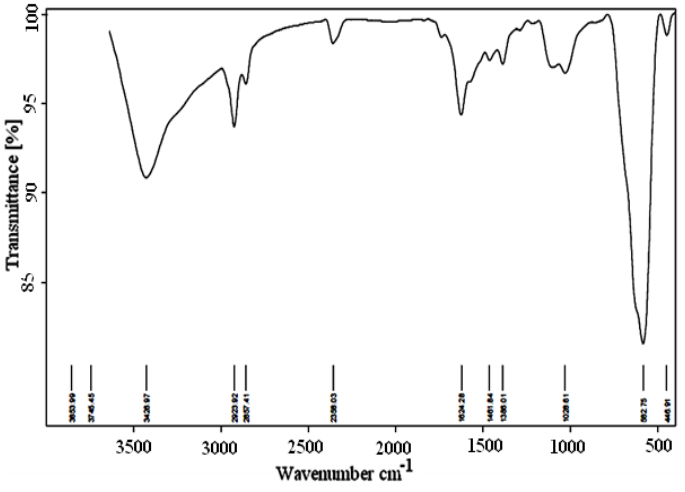

Fig. 1. FT-IR spectra of the magnetic nanoparticles of Fe_3O_4 before immobilization of GOx.

3.1 Study of the synthesized Fe_3O_4 structure

Fourier Transform Infrared Spectroscopy (FT-IR) was used for determination of functional groups of the synthesized Fe_3O_4 (Fig. 1). The FT-IR bands at law wave numbers, between 500 cm^{-1} and 700 cm^{-1} were related to vibrations of Fe-O bonds of iron oxide [35]. The characteristic absorption peak of Fe_3O_4 lies at 582.75 cm^{-1} [35]. The absorption bands near 3426.96 cm^{-1} are related to hydroxyl groups on the surface of Fe_3O_4. Catalytic activity of Fe_3O_4 for H_2O_2 decomposition can be affected by the presence of surface -OH groups on it and the rate of reaction increases logarithmically with increase in -OH groups. Presence of surface -OH groups on Fe_3O_4 indicate, Fe_3O_4 can be decompose H_2O_2 produced in glucose oxidation reaction. After immobilezation of GOx on the support, the new absorption bands at 1637.56 cm^{-1} and 3275.92 cm^{-1} were appeared (Fig. 2). These bands are related to the NH_4^+ bending vibration and N-H band respectively which are found in the enzyme structure and confirm the enzyme immobilization.

The crystalline structure of Fe_3O_4 sample was examined by X-ray diffraction. XRD pattern of the Fe_3O_4 was scanned between 20^o and 70^o in 2θ. The XRD pattern of the Fe_3O_4 sample has been depicted at Fig.3. Six distinct peaks are seen at 2θ=29.2, 35.1, 42.9, 52.2, 55.0, 61.8. Sharp peaks in the corresponding XRD pattern demonstrate high crystalline structure of Fe_3O_4. From the line broadening of corresponding X-ray diffraction peaks, the crystallite sizes of magnetic Fe_3O_4 nanoparticles were estimated using the Williamson-Hall equations [41,42].

The Williamson-Hall's equation for crystalline size calculation is:

$$\beta\cos\theta = K\lambda / D + \varepsilon\sin\theta \qquad (2)$$

where, λ is the wavelength of the X-ray radiation (1.54056 nm), K is a constant of diffraction taken as 0.89, D is crystal size, θ is the diffraction angle, β is the full width at half maximum corrected for instrument broadening. A plot of βcosθ against sinθ yields the crystal size from the slope 0.89λ/D. The crystal sizes were obtained approximately 19 nm for the sample by Williamson-Hall equations.

The morphology of the Fe_3O_4 sample was studied by the transmission electron microscopy (TEM). As can be seen from Fig. 4, it is found that the size distribution of the magnetic nanoparticles is from 18 nm to 20 nm (Fig.4), which is coordinated with XRD.

The N_2 adsorption–desorption measurement using liquid N_2 (temperature of −196°C) was performed to determine specific surface area of the Fe_3O_4 sample. The specific surface area of the sample calculated from the nitrogen isotherm using the BET method, was 62.7m2/g. This property indicates that Fe_3O_4 has enough specific surface area which is necessary for catalytic role of Fe_3O_4 in H_2O_2 decomposition and adsorption of the enzyme in the support.

To investigate the magnetic properties of Fe_3O_4 nanoparticles, magnetic measurement was performed. Fig. 5 shows the magnetization curve of the Fe3O4 nanoparticles. The saturation magnetization of Fe_3O_4 nanoparticles was obtained 68.8emu/g in 8 KOe. With the large saturation magnetization, the magnetic nanoparticals can be separated from the reaction medium rapidly and easily in a magnetic field. In addition, as

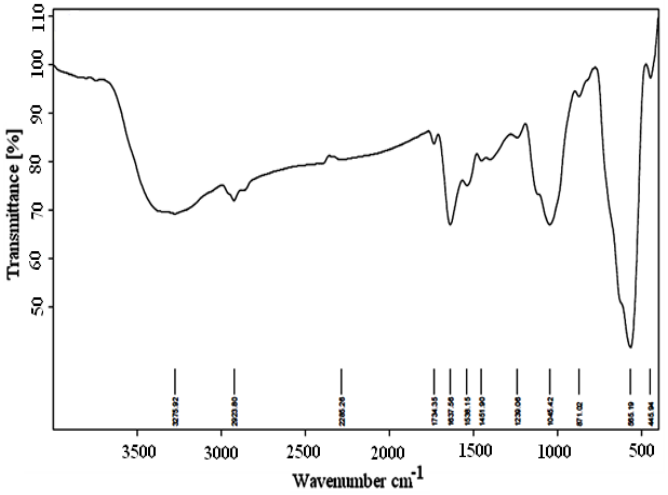

Fig. 2. FT-IR spectra of the magnetic nanoparticles of Fe_3O_4 after immobilization of GOx.

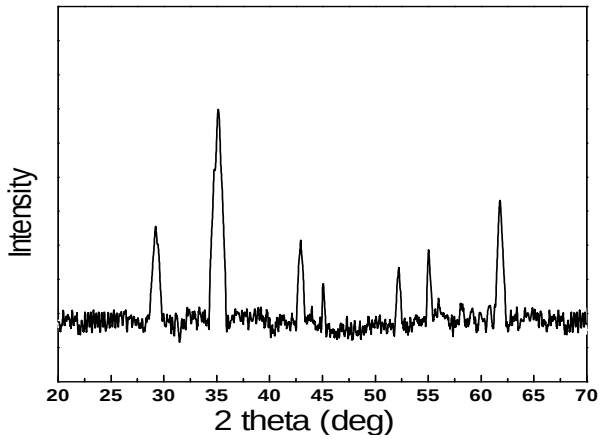

Fig. 3. X-ray diffraction of the synthesized Fe_3O_4.

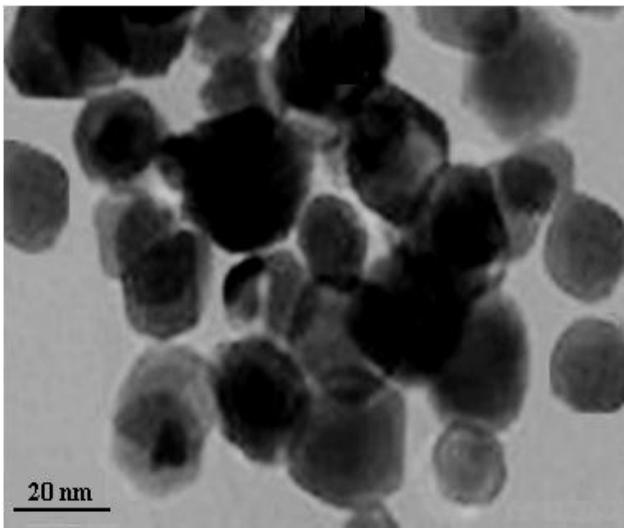

Fig. 4. TEM image of the synthesized Fe_3O_4.

shown in fig. 5, there was no hysteresis in the magnetization with both remanence and coercivity being zero, indicating that these magnetic nanoparticles exhibit a superparamagnetic behavior [43]. Fig. 5 also, illustrates after GOx immoblition there is little decreasing in the saturation magnetization.

3.2 Activity of the immobilized Enzyme

To compare the performance of the free and immobilized GOD, the deoxygenation activity in the first min was used as an indicator of the initial activities expressed as IAFE (U/ml) and IAIME (U/g), respectively. The amount of enzyme immobilized was determined from the difference between the initial and final enzyme activities in the reaction media. Although very small, the amount of enzyme desorbed into the washing solution was also accounted in calculating the immobilization efficiency. Percentage of immobilization was obtained 78%. The same amount of enzyme which was attached to the 1 g of support was chosen for 1 ml of soluble enzyme to compare their deoxygenation activity. The results show IAIME was decreased to 640 U/g in compare to the IAFE which was equal to 780 U/ml respectively. This may be due to deactivation of several active sites of the enzyme because of perturbation in the conformation of protein molecules adsorbed on the support and the hindrance in accessing some enzyme active sites after adsorption by substrate molecules.

3.3 Strength of enzyme adsorption on the support

The GOx-Fe_3O_4 catalyst was washed 30 times with tap water to study the strength of the immobilized GOx on the support (Fig.6 shows the results). Reduction of the activity per each test was insignificant so that after 30 wash test, only 14% of the initial activity was reduced which in turn indicates there is a strong interaction between GOx molecules and the support.

3.4 GOx- Fe_3O_4 catalyzed deoxygenation of water

Biological deoxygenation was carried out in a 150 mL bioreactor in a shaking incubator by glucose-GOx (the enzyme in free and immobilized states) at 35ºC. As can be seen from Fig 7, removal of DO from 150 ml of tap water with 6.9 mg/l O_2, catalyzed by the 0.15g hybrid catalyst in the presence of twice stochiometric amount of glucose was completed during 4 min and 20s. Deoxygenation by free GOx and catalase in the same conditions was completed during 3 min and 33s. This indicates DO removal rate not decreases significantly when homogeneous system turns to heterogeneous system.

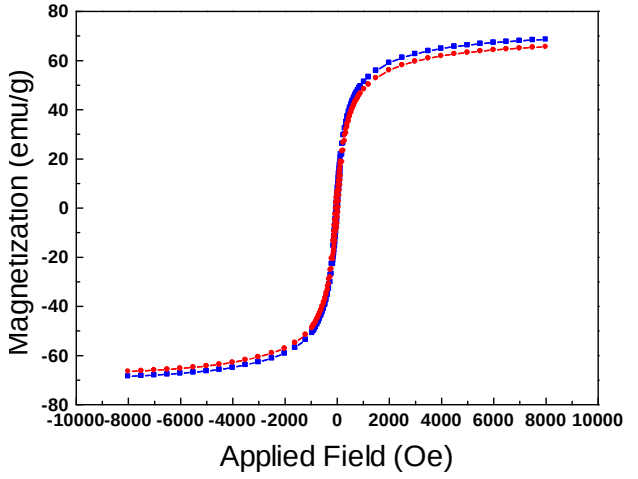

Fig. 5. Magnetization curves of the synthesized Fe3O4 nanoparticles (—▲—) before GOx immobilization (—●—) after GOx immobilization.

4 Conclusion

Supermagnetic Fe_3O_4 nanoparticles were synthesized to obtain high surface area to achieve high load of enzyme on it. Characterization of as-prepared Fe_3O_4 was studied. The crystal sizes of the magnetic nanoparticles of Fe_3O_4 were calculated approximately 19 nm by XRD. The saturation magnetization value of Fe_3O_4 nanoparticles was obtained 68.8 emu/g, which

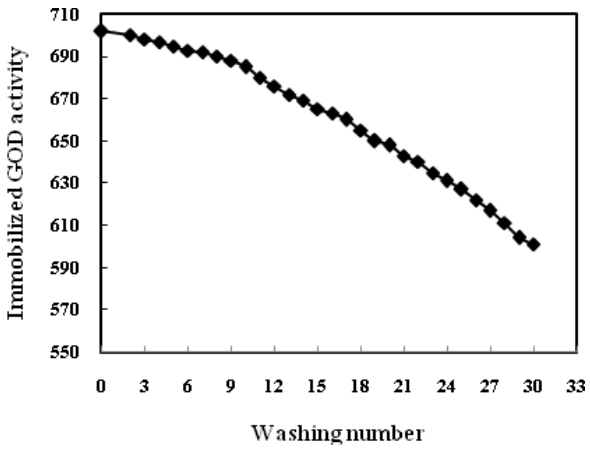

Fig. 6. Strength of enzyme adsorption on the synthesized Fe_3O_4 support.

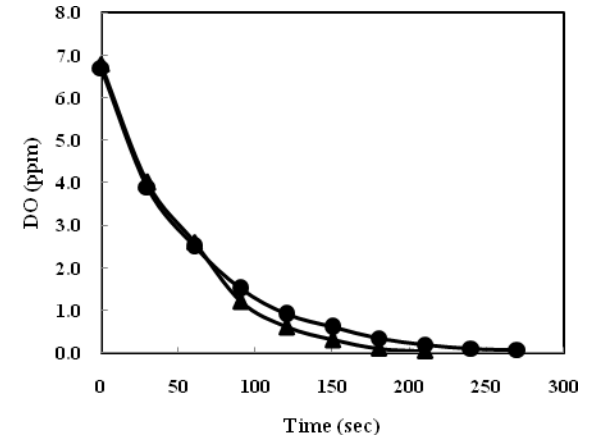

Fig. 7. Removal of DO (6.9 mg/l O2) from 150 ml water in a bioreactor (150 r/min) at 35ºC in shakerincubator using glucose-free GOD + catalase (—▲—), glucose- immobilized GOD (—●—). Glucose concentration was twice stochiometric. Activity of catalase and GOD were 200 U and 100 U in a free state and 0.15g immobilized GOD was used.

indicates Fe_3O_4 nanoparticles are supermagnetic. Fe_3O_4 nanoparticles specific surface area was 62.7m²/g, which is appropriate for enzyme immobilization. Removal of dissolved oxygen from water was investigated using a biological method. Glucose used as a biological matter that reacts with DO in the presence of GOx as a biocatalyst. Products of this reaction are H_2O_2 and Gluconic acid. All of the materials (reactants and products) are safe and it is the important aspect of our study. Gluconic acid is an oxygen scavenger so amplify removal of DO. H_2O_2 is decomposed by Fe_3O_4 as a catalyst. Fe_3O_4 has two roles, one as a support, and another as a catalyst for H_2O_2 decomposition. Removal of DO from tap water catalyzed by the hybrid catalyst in the presence of twice stochiometric amount of glucose was carried out with good rate and noticeable efficiency.

REFERENCES

[1] A. H. Lu, E. L. Salabas, and F. Schuth, "Magnetic nanoparticles: Synthesis, protection, functionalization, and application," Angew. Chem. Int. Edit, vol. 46, no. 8, pp. 1222–1244, February. 2007.

[2] C. S. S. R. Kumar, Biofunctionalization of Nanomaterials. Vol I, Wiley-VCH: Weinheim, pp. 72–98, 2005.

[3] A. Hutten, D. Sudfeld, I. Ennen, G. Reiss, W. Hachmann, U. Heinzmann, K. Wojczykowski, P. Jutzi, W. Saikaly, and G. Thomas, "New magnetic nanoparticles for biotechnology," J. Biotechnol, vol. 112, no. 1-2, pp. 47–63, August. 2004.

[4] A. K. Gupta, and M. Gupta, "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications," Biomaterials, vol. 26, no. 18, pp. 3995–4021, June. 2005.

[5] R. G. L. Audran and A. P. Huguenard, "Magnetic recording elements containing transparent recording layer," U.S. Pat. No. 4302523, May. 1981.

[6] L. J. Daniel, A. K. Thorek, J. C. Chen, and T. Andrew, "Superparamagnetic Iron Oxide Nanoparticle Probes for Molecular Imaging," Annals of Biomedical Engineering, vlo. 34, no. 1, pp. 23-38, February. 2006.

[7] T. Kobayashi, A. Saito and H. Tsuji, "Magnetic refrigerator," U.S. Pat. 7536866, May. 2009.

[8] C. Pan, B. Hu, W. Li, Y. Sun, H. Ye, X. Zeng, "Novel and efficient method for immobilization and stabilization of β-d-galactosidase by covalent attachment onto magnetic Fe_3O_4–chitosan nanoparticles," J. Mol. Catal. B: Enzym, vol. 61, no. 3-4, pp. 208–215, July. 2009.

[9] C. Ingrosso, M. Striccoli, A. Agostiano, E. Sardella, S. Keller, G. Blagoi, A. Boisen, and M. L. Curri, "Surface Functionalization of Micro Mechanical Cantilever Sensors by Organic Capped TiO2 and Fe2O3 Nanocrystals," Procedia Chemistry vol. 1, no. 2, pp. 32–35, April. 2009.

[10] L. Zhu, P. Chen, Y. Guo, Y. Song, X. Xue and X. Cao, "Ternary ZnO/ZnS/γ-Fe_2O_3 hollow sphere with surface hole: Microwave-enhanced rapid synthesis, bifunctional property, and immobilization of serum protein," Colloid Surf. A: Physicochem Eng. Aspects, vol. 360, no. 1-3, pp. 111–119, May. 2010.

[11] X. Zhang, R. Guan, D. Wu and K. Chand, "Enzyme immobilization on amino-functionalized mesostructured cellular foam surfaces, characterization and catalytic properties," J. Mol. Catal. B: Enzym, vol. 33, no. 1-3, pp. 43–50, May. 2005.

[12] T. Georgelin, B. Moreau, N. Bar, D. Villemin, V. Cabuil and O. Horner, "Functionalization of γ-Fe_2O_3 nanoparticles through the grafting of an organophosphorous ligand," Sens. Actuat. B, vol. 134, no. 2, pp. 451–454, September. 2008.

[13] D. Ansil, L. Katja, N. Mayumi, W. Seung, C. S. Chang, V. P. M. Kurikka, A. U. Shafi, M. Cowman, and A. G. Richard, "Activity of Candida rugosa Lipase Immobilized on γ-Fe_2O_3 Magnetic Nanoparticles," J. Am. Chem. Soc., vol. 125, no. 7, pp. 1684-1685, February. 2003.

[14] W. Aehle, Enzymes in industry: production and applications, Wiley, pp. 50-100, 2007.

[15] R. Dave and D. Madamwar, "Entrapment of lipase in polymer of polyvinyl alcohol-boric acid for esterification in organic media," Indian J. biotechnol, vol. 5, no. 3, pp. 368-372, July. 2006.

[16] D. Rolf Schmid, Carrier-bound immobilized enzymes principles, applications and design, Wiley-VCH, pp. 110-130, 2005.

[17] P. Rani, M. Sharma, V. Kumar and C. S. Pundir, "Immobilization of amylase onto arylamine glass beads affixed inside a plastic beaker: Kinetic properties and application," Indian J. biotechnol, vol. 6, no. 2, pp. 230-233, April. 2007.

[18] M. Sharma, V. Kumar and C. S. Pundir, "Immobilization of porcine pancreas lipase onto free and affixed arylamine glass beads and its application in removal of oil stains," Indian J. biotechnol, vol. 7, no. 3, pp. 328-332, July. 2008.

[19] S. Libertino, A. Scandurra, V. Aiello, F. Giannazzo, F. Sinatra, M. Renis, and M. Fichera, "Layer uniformity in glucose oxidase immobilization on SiO_2 surfaces," Appl. Surf. Sci, vol. 253, no. 23, pp. 9116–9123, May. 2007.

[20] M. Tasviri, H. A. Rafiee-Pour, H. Ghourchian, and M. R. Gholami, "Amine functionalized TiO_2 coated on carbon nanotube as a nanomaterial for direct electrochemistry of glucose oxidase and glucose biosensing," J. Mol. Catal. B: Enzym, vol. 68, no. 2, pp. 206–210, February. 2011.

[21] L. Zhu, R. Yang, J. Zhai, and C. Tian, "Bienzymatic glucose biosensor based on co-immobilization of peroxidase and glucose oxidase on a carbon nanotubes electrode," Biosens. Bioelectron, vol. 23, no. 4, pp. 528–535, November. 2007.

[22] S. Zhang, N. Wang, Y. Niu, and C. Sun, "Immobilization of glucose oxidase on gold nanoparticles modified Au electrode for the construction of biosensor," Sens. Actuators. B, vol. 109, no. 2, pp. 367–374, July. 2005.

[23] J. Landoulsi, K. El Kirat, C. Richard, R. Sabot, M. Jeannin, and S. Pulvin, "Glucose oxidase immobilization on stainless steel to mimic the aerobic activities of natural biofilms," Electrochim. Acta, vol. 54, no. 1, pp. 133–139, December. 2008.

[24] C. Sisak, Z. Csanádi, E. Rónay, and B. Szajáni, "Elimination of glucose in egg white using immobilized glucose oxidase," Enzyme Microb. Technol, vol. 39, no. 5, pp. 1002–1007, September. 2006.

[25] B. Tahsin and S. Ç. Serdar, "Performance of immobilized glucoamylase in a magnetically stabilized fluidized bed reactor (MSFBR)," Enzyme Microb. Technol, vol. 26, no. 1, pp. 28–33, January. 2000.

[26] T. Bahar and S. S. Celebi, "Characterization of glucoamylase immobilized on magnetic poly (styrene) particles," Enzyme Microb. Technol. Vol. 23, no. 5, pp. 301–304, October. 1998.

[27] B. R. Pieters and G. Bardeletti, "Enzyme immobilization on a low-cost magnetic support: Kinetic studies on immobilized and coimmobilized glucose oxidase and glucoamylase," Enzyme Microb. Technol. Vol. 14, no. 5, pp. 361–370, May. 1992.

[28] X. Q. Liu, Y. P. Guana, R. Shen, and H. Z. Liu, "Immobilization of lipase onto micronsize magnetic beads," J. Chromatogr B, vol. 822, no. 1-2, pp. 91–97, August. 2005.

[29] A. Karimi, F. Mahdizadeh, D. Salari, and A. Niaei, "Bio-deoxygenation of water using glucose oxidase immobilized in mesoporous MnO_2," Desalination, vol. 275, no. 1-3, pp. 148-153, July. 2011.

[30] I. B. Butler, M. A. A. Schoonen, and D. T. Rickard, "Removal of dissolved water: a comparison of four common techniques," Talanta, vol. 41, no. 2, pp. 211-215, February. 1994.

[31] V. Sinha and K. Li, "Alternative methods for dissolved oxygen removal from water: a comparative study," Desalination, vol. 127, no. 2, pp. 155 – 164, February. 2000.

[32] J. Shao, H. Liu, and Y. He, "Boiler feed water deoxygenation using hollow fiber membrane contactor," Desalination, vol. 234, no. 1-3, pp. 370–377, December. 2008.

[33] A. Ito, K. Yamagiwa, M. Tamura, and M. Furusawa, "Removal of dissolved oxygen using non-porous hollow-fiber membranes," J. Membr. Sci. vol. 145, no. 1, pp. 111-117, June. 1998.

[34] X. Tan, G. Capar, and K. Li, "Analysis of dissolved oxygen removal in hollow fibre membrane modules: effect of water vapour," J. Membr. Sci. vol. 251, no. 1-2 pp. 111-119, April. 2005.

[35] J. S. Moon, K. K. Park, J. H. Kim, and G. Seo, "Reductive removal of dissolved oxygen in water by hydrazine over cobalt oxide catalyst supported on activated carbon fiber," Appl. Catal. A vol. 201, no. 1, pp. 81-89, June. 2000.

[36] K. Li, and X. Tan, "Development of membrane-UV reactor for dissolved oxygen removal from water," Chem. Eng. Sci. vol. 56, no. 17, pp. 5073-5083, September. 2001.

[37] R. V. D. Vaart, I. Petrova, V. Lebedeva, V. Volkov, D. Kochubey, and G. Tereshchenko, "In-situ application of catalytic phase to commercial membrane contactor for removal of dissolved oxygen from water," Desalination, vol. 199, no. 1-3, pp. 424-425, November. 2006.

[38] X. Tan, and K. Li, "Investigation of novel membrane reactors for removal of dissolved oxygen from water," Chem. Eng. Sci. vol. 55, no. 7, pp. 1213-1224, April. 2000.

[39] M. Ozmen, K. Can, G. Arslan, A. Tor, Y. Cengeloglu, and M. Ersoz, "Adsorption of Cu(II) from aqueous solution by using modified Fe_3O_4 magnetic nanoparticles," Desalination, vol. 254, no. 1-3, pp. 162-169, May. 2010.

[40] S. B. Bankar, M. V. Bule, R. S. Singhal, and L. Ananthanarayan, "Glucose oxidase - an overview," Biotechnol. Adv, vol. 27, no. 4, pp. 489–501, April. 2009.

[41] Z. Shu and S. Wang, "Synthesis and characterization of magnetic nanosized Fe_3O_4/MnO_2 composite particles," J. Nanomat, vol. 2009, no. 5, pp. 1-5, October. 2009.

[42] B. D. Cullity and S. R. Stock, Elements of X-ray diffraction, Printice Hall: New Jersey, pp.388-390, 2001.

[43] Y. Cui, Y. Li, Y. Yang, X. Liu, L. Lei, L. Zhou, and F. Pan, "Facile synthesis of amino-silane modified superparamagnetic Fe_3O_4 nanoparticles and application for lipase immobilization," J. Biotechnol, vol. 150, no. 1, pp. 171–174, October. 2010.