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Bio-deoxygenation of water using glucose oxidase immobilized in mesoporous MnO_2

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ABSTRACT

In this research, removal of water dissolved oxygen reduced by glucose and catalyzed by glucose oxidase (GOD) was the focus of attention. In order to maintain the enzyme in repeated or continuous runs, its immobilization in the porous manganese dioxide support was carried out. In addition to enzyme immobilization support, manganese dioxide also plays catalytic role in the decomposition of hydrogen peroxide produced during the glucose oxidation reaction. In so doing synthesized porous MnO_2 was applied with the specific surface area of 176.93 m^2/g and total pore volume of 0.47 ml/g. The activity of hybrid GOD–MnO_2 biocatalyst in the removal of dissolved oxygen was achieved to be 700 U/g. Deoxygenation of 150 ml of tap water with 6.50 mg/l O_2 which was catalyzed by the 0.15 g hybrid catalyst in the presence of twice stochiometric amount of glucose was completed in less than 4 min at 35 °C.

1. Introduction

In many industries water deoxygenation is a necessary step because dissolved oxygen (DO) causes some problems such as pitting corrosion [1–3]. 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 [4–6]. Chemical methods on the other hand include use of a reducing agent such as hydrazine, sodium sulfite and hydrogen in different procedures [7–10]. All of them have some defects from different aspects such as low efficiency, toxicity, economical problems and hard working conditions.

Biological materials such as glucose have the capability of being chemically oxidized by DO, but in that case the rate of reaction becomes very low. Oxidases are enzymes that catalyze the oxidation–reduction reactions, having that in mind they can easily catalyze the DO reduction to the H_2O_2 or H_2O in the presence of suitable substrates. For example, glucose reacts with O_2 to produce gluconic acid and H_2O_2.

Enzymes are used as biocatalysts in the many chemical, pharmaceutical and food industries and as specific ligands in clinical and chemical analysis [11]. Having the ability to make high-cost enzymes reusable and stable, enzyme immobilization has attracted a great deal of attention. Heterogeneous processes are generally considered to be more economical and profitable than homogeneous ones at industrial scale due to significant simplification and cost reduction of the whole technological cycle. Heterogeneous biocatalysts are prepared by immobilization of active substances (enzymes and cells of microorganisms) on water-insoluble supports to form the basis for development of novel modern bioprocesses. There are various techniques such as physical adsorption, covalent binding, entrapment, and cross linking to enzyme immobilization [12]. Every technique has some advantages and drawbacks; Immobilization by physical adsorption is caused by interaction between enzyme and support such as electrostatic and hydrophobic interactions or hydrogen bonds. Physical adsorption is limited by the tendency of enzyme to desorb from the support. In the last few decades, adsorptive enzyme immobilization techniques have been intensively studied, because of some advantages including reversibility, simplicity, which enables enzyme immobilization under mild conditions [13].

Varieties of supports have been used for immobilization of GOD such as Al_2O_3, SiO_2, glassy carbon, Au nanoparticals, stainless steel, Amberlite UP 900 anion exchange resin, etc. for different goals [14–21]. Usually immobilized GOD has been studied as a bio-anode for glucose fuel cells, for example, immobilization of GOD on carbon paper electrode modified with conducting copolymer of 3-methythiopene and thiophene 3-acetic acid has been done [22]. Another common field of application of immobilized GOD relates to glucose biosensor. For this mean GOD has been immobilized on different supports such as colloidal gold modified carbon paste electrode [23], Au electrode modified with gold nanoparticals [19,24], amine functionalized TiO_2 coated on carbon nanotube [17], ZnO nanotubes [25], carbon nanotubes [18], and etc. MnO_2 nanoparticals were successfully used in the fabrication of electrochemical biosensors [26,27]. All of the above mentioned supports are suitable for its unique applications.

The main aim of this study is the biological oxygen removal from water catalyzed by glucose oxidase by using oxidation of glucose. Immobilization of the enzyme on mesoporous MnO_2 was carried out to take it suitable for use in continuous process. In addition to the large specific surface area of the mesoporous MnO_2 which is necessary

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for the high load of enzyme, this material also plays catalyst role in the decomposition of hydrogen peroxide produced in glucose oxidation reaction. Deoxygenation from water by the new method also has been compared with conventional chemical oxygen scavengers.

2. Experimental

2.1. Materials

Glucose oxidase (GOD) type II (EC 1.1.3.4, 25 U/mg, from Aspergillus niger), β-D-(+)-glucose, sodium acetate, acetic acid, $MnCl_2$, $KMnO_4$, 2,2′-Azino-di-[3-ethylbenzthiazolin-sulfonate] (ABTS), sodium sulfite, hydrazine, and CCl_4 in the analytical grade were obtained from Sigma Aldrich.

2.2. Preparation of the mesoporous MnO_2

30 ml of 1 M $MnCl_2$ was added into 80 ml CCl_4 solution and 5 min later, after the separation of two phases, the obvious CCl_4/H_2O interface was obtained. Then 40 ml of 0.50 M $KMnO_4$, by two drops per second, through the CCl_4 layer reached the CCl_4/H_2O interface drop by drop (Fig. 1.). At first, a brown material (MnO_2) appeared at the interface as soon as drops reached it. Reaction system was remained in a static condition for 48 h, during this time the whole $MnCl_2$ solution slowly react with $KMnO_4$ through the redox to convert into brown MnO_2. Then, the collected MnO_2 was washed repeatedly with deionized water and then with absolute ethanol for three times in order to remove the impurities. Finally, the obtained solid was dried at 150 °C in air for 12 h [28].

2.3. Characterization of the synthesized MnO_2

Fourier Transform Infrared Spectroscopy (FT-IR) was recorded by a Tensor 27 Model (Bruker German) spectrophotometer using KBr pellets. The X-ray diffraction (XRD) pattern of the as-prepared sample was determined using a D5000 diffractometer (Siemens German) with Cu K_α radiation source (λ = 1.54056 Å). Morphology of the sample was examined by scanning electron microscopy (SEM) Leo 1455 VP Model with 10 kV voltage. Surface area and pore volume of the prepared MnO_2 were obtained through the Brunauer–Emmett–Teller (BET) with micromeritics Gemini 2375 (USA) analyzer.

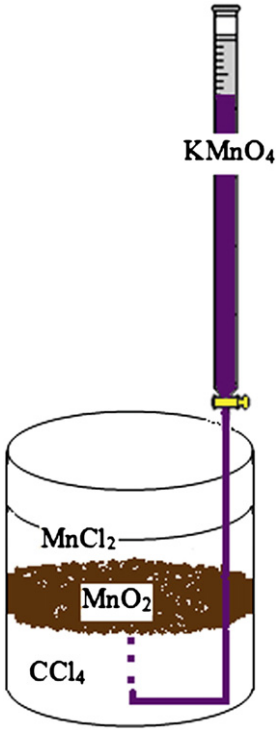

Fig. 1. Schematic representation of mesoporous MnO_2 synthesis.

2.4. Assay of GOD activity

GOD 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 [20]. One unit of catalyst activity (U) is defined as the amount of GOD required to consume 1 μmol substrate in 1 min at 25 °C [29]. In the all experiments temperature was controlled by an electronically thermostat water bath.

2.5. Immobilization of GOD

1 g of the mesoporous MnO_2 was dispersed in 10 ml of 140 U/ml GOD solution in acetate buffer at pH 5.50 with stirring in a magnetic stirrer for 1 h at room temperature. The experiments showed that the use of higher dosages of GOD do not have more effect on the amount of enzyme immobilization in the support (the results were not shown here). The obtained hybrid catalyst was separated from the solution by filtration and was stored at 4 °C until use.

2.6. Assay of the GOD–MnO_2 hybrid catalyst activity

Glucose oxidase immobilized in MnO_2 directly converts glucose to gluconic acid and H_2O as depicted in (Eq. (1)). Glucose solution (0.10 M) was filled into a precisely sealed mixed reactor. 0.10 g GOD–MnO_2 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 1 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 1 μmol O_2 per min at 25 °C.

$$\beta\text{-D-glucose} + \frac{1}{2}O_2 \xrightarrow{\text{GOD-MnO}_2} \text{D-gluconicacid} + H_2O \quad (1)$$

3. Results and discussion

3.1. Study of the synthesized MnO_2 structure

Fourier Transform Infrared Spectroscopy was used for the determination of functional groups of the synthesized mesoporous MnO_2. As it can be seen from Fig. 2a, absorption bands at 525 cm^{-1} and 572 cm^{-1} are related to Mn—O and a band at 3196 cm^{-1} indicates tension vibration of —OH. A band at 1619 cm^{-1} is ascribed to bending vibration of —OH with Mn atoms [30,31]. Catalytic activity of MnO_2 for H_2O_2 decomposition can be affected by the presence of surface —OH groups on it so the rate of reaction increases logarithmically with increase in —OH groups [32]. After immobilization of GOD on the support, the new absorption bands at 1639.4 cm^{-1} and 3288.4 cm^{-1} were appeared (Fig. 2b). These bands are related to the NH_4^+ bending vibration and N—H band respectively which are found in the enzyme structure.

X-ray diffraction pattern of the MnO_2 which was recorded at 2θ = 10–70° has been depicted in Fig.3. Five distinct peaks are seen at 2θ = 25.1, 32.0, 37.1, 42.5, and 57.1. A peak at 2θ = 32 related to γ-MnO_2, peaks at 2θ = 25.1, 37.1 represents α-MnO_2, and peaks at