Journal of Industrial and Engineering Chemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Cyclic-carbonate functionalized polymer brushes on polymeric microspheres: Immobilized laccase for degradation of endocrine disturbing compounds

Gulay Bayramoglu[a,b,*], Bunyamin Karagoz[c], M. Yakup Arica[b]

[a] Biochemical Processing and Biomaterial Research Laboratory, Gazi University, 06500 Teknikokullar, Ankara, Turkey
[b] Department of Chemistry, Gazi University, 06500 Teknikokullar, Ankara, Turkey
[c] Istanbul Technical University, Department of Chemistry, Maslak 34469, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 27 July 2017
Received in revised form 20 October 2017
Accepted 17 November 2017
Available online xxx

Keywords:
Immobilized laccase
Endocrine disturbance
Packed bed reactor
Environmental biotechnology
Nano-structured materials

ABSTRACT

A novel support material containing cyclic carbonate group for facile enzyme immobilization was synthesized from [poly(styrene-co-divinylbenzene)-graft-poly(glycidyl methacrylate)] "[PS-co-DVB-g-P(GMA)]" microsphere under carbon dioxide atmosphere. It was named as (poly(styrene-co-divinylbenzene)-graft-poly(cyclic-carbonate methacrylate)] "[PS-co-DVB-g-P(CCMA)]" microsphere. Laccase was covalently immobilized using the microspheres carrying cyclic epoxy and cyclic carbonate groups. The amount of enzyme loading on the cyclic carbonate groups of the microspheres was 47.8 mg/g. The immobilized enzyme was used for degradation of Bisphenol A, and Congo Red dye in packed bed reactor. The immobilized laccase preserved its initial activity about 67.5% and 93.0% for degradation of Bisphenol A and Conge Red, respectively.

Introduction

Several organic pollutants such as dyes, pesticide, and drugs have been reported as water contaminants. In textile industry, up to 50% of the used dyes are discharged in industrial effluent after dyeing process [1]. Chemical and textile industrial effluents contain organic molecules that can be toxic, mutagenic or carcinogenic for living organisms [2–5]. Several methods have been proposed for removal of the organic pollutants from wastewaters such as microbial and enzymatic degradation [6–8] adsorption/biosorption [9–11], and chemical processes [12,13]. For oxidative degradations of organic pollutants several oxidative enzymes have been used such as laccase, tyrosinase, manganese peroxidase, horseradish peroxidase etc. Among them, laccase can oxidase a wide spectrum of aromatic organic compounds in the presence of O_2 [14–17].

Laccases (E.C.1.10.3.2., oxygen oxidoreductase) from various white rot fungi can oxidize several aromatic hydrocarbons such as synthetic dyes, pesticides and lignin related compounds [18,19]. These enzymes are belonging to the multi-copper oxidases family commonly found in plants, insects, fungi and bacteria. The most known laccase producers are nearly all wood-rotting fungi, such as *Trametes versicolor*, *Trametes hirsuta*, *Phanerochaete chrysosporium, and Bjerkandera adusta*. Therefore, laccase has many possible applications, including textile effluent discoloration, bio-bleaching of paper pulp, and the detoxification of different xenobiotics [20,21]. Immobilization of enzymes and microorganism on insoluble support materials is accepted as useful approach for improvement of their reusability and stability [22–25]. The selection of support material is the most important key factor for immobilization of enzymes. Various inorganic and organic support materials for immobilization of enzyme have been used such as polymeric films [26–29], chitosan/acrylate composite, functionalized magnetic nano-particles [30,31], magnetic silica nanoparticles [32], zeolite/polymer and synthetic polymeric beads [3,16]. On the other hand, fibrous polymer grafted support materials may be more effective carriers for enzyme immobilization, because of having large surface areas, and permitting large amount of enzyme attachment.

An addition, the fixed enzyme on the fibrous polymer can be dispersed in the reaction medium likes a free enzyme. Therefore, immobilization of enzyme on fibrous polymer grafted support can provide high retained enzyme activity, high enzyme loading and fast enzymatic reaction kinetics compared with other supports [27,33]. The fibrous polymer on the shell can be functionalized with

* Corresponding author at: Department of Chemistry, Gazi University, 06500 Teknikokullar, Ankara, Turkey.
E-mail address: gbayramoglu@hotmail.com (G. Bayramoglu).

https://doi.org/10.1016/j.jiec.2017.11.028
1226-086X/

different reactive groups, such as epoxy, hydroxyl, amine, thiol, carboxyl, chloride, bromide and cyclic carbonate etc. [26,34]. These functional groups are principally used for the immobilization of the biological molecules. The cyclic carbonate carrying polymers are mostly used in the production of thermoset plastics, and also are used for the coating of polyurethane based materials via the carbonate–amine reaction. The acrylate polymers carrying the epoxy group are the most suitable support materials for large scale immobilization of enzymes and other proteins, and also permit derivatization of different functional groups. The SI-ATRP method is one of the effective derivatization approach to graft support surface with functional fibrous polymer [3,26,35]. An addition, the SI-ATRP method allows the preparation of well-defined polymer grafting on the various materials with different functional groups [36,37]. Moreover, the coating of a support with fibrous polymer may provide a homogenous immobilization conditions, and also can provide easy substrate and product diffusion from solid to solution or vice versa.

As well known, the laccases have been used in the several studies to treat wastewaters containing aromatic organic pollutants, and it has high potential in this area. For this reason, *T. versicolor* laccase was selected for covalent immobilization of enzyme via the direct cyclic carbonate coupling reaction on the [PS-co-DVB-g-P(CCMA)] microspheres. The major objective of the study was to obtain the maximum degradation capability of the immobilized laccase for some model endocrine disturbing chemicals by optimizing the immobilization process factors such as immobilization time, and temperature, and initial amount of enzyme in the medium. It would been showed that the results can be valuable in facilitating the use of cyclic carbonate coupling reaction for immobilization of various oxidases for practical applications in the field of environmental protection. Additionally, the cyclic carbonate containing polymeric structure, in which the laccase enzyme directly immobilized via covalent bonding, has not been used according to the literature scanning. In order to achieve these goals, firstly, the PS-co-DVB microspheres were produced via suspension polymerization. Then, the microspheres were modified with an ATRP initiator, and then P(GMA) fibrous chains were introduced as a reactive group carrying polymer via SI-ATRP methods. Secondly, the preparation of [PS-co-DVB-g-P(CCMA)] from [PS-co-DVB-g-P(GMA)] microspheres was achieved using carbon dioxide and LiBr as catalyst in dimethylformamide. The cyclic carbonate group of the support was used for covalent immobilization of laccase using the epoxy group containing counterpart as a control system. This study also includes a systematic characterization of the morphology and the chemical composition of the [PS-co-DVB-g-P(GMA)] and [PS-co-DVB-g-P(CCMA)] microspheres using ATR-FTIR spectroscopy, SEM, BET, and analytical methods. In addition, the thermal and storage stabilities and reusability of the immobilized laccase were also tested to show the advantage of immobilization of laccase via cyclic carbonate group compared with immobilization via epoxy group. Finally, laccase enzyme immobilized systems were used for enzymatic degradation of two different endocrine disruptors (i.e., Bisphenol A and Congo Red dye) in aqueous medium. The complete degradation time of the Congo Red dye was determined using UV–vis spectrophotometer and MALDI-ToF-MS studies.

Experimental sections

Materials and methods

Laccase (EC 1.10.3.2): *p*-diphenol:dioxygen oxidoreductase; (*T. versicolor* about 20 U/mg), 4-hydroxy-3,5-dimethoxyhydroxy-benzaldehyde (syringaldazine), CuBr, bromoacetyl bromide, triethylamine, tetrahydrofuran, aqueous ammonia, α,α′-azobisizobutyronitrile (AIBN) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Chem. Co. The initiator AIBN (% 98) was purified by recrystallization from methanol. The ATRP ligand, H-TETA (1, 1, 4, 7, 10, 10-hexakis [hexyl1, 4, 7, 10-tetraazadecane]) was prepared by action of triethylene tetramine upon 1-bromohexane as described before [21]. The monomers, (i.e., divinylbenzene (DVB), styrene (S) and glycidylmethacrylate (GMA)), mercuric acetate, and Amberjet 1200 Na were supplied from Sigma-Aldrich Co. The former two monomers (i.e., divinyl-benzene and styrene) was washed with NaOH aqueous solution (4.0%) to remove inhibitor prior to use. The later "glycidyl methacrylate" was distilled over CaH_2 under reduced pressure before use. All other chemicals were of analytical grade and supplied from Merck (Darmstadt, Germany) and all these chemicals were used as purchased.

Synthesis of poly(glycidyl methacrylate) brushs on PS-co-DVB microspheres by SI-ATRP method

Polystyrene-divinyl benzene, PS-co-DVB, as the starting polymeric microspheres was synthesized by suspension polymerization using styrene and divinyl benzene monomers in the presence of arabic gum stably, as in our work described in the literature [35]. The synthesized microspheres were dried and sieved, and the 210–422 μm size fraction was used in further reactions. ATRP initiator sites, bromoacetyl groups were then tethered to the surface of the PS-co-DVB microspheres by acetoxy mercury method as reported in the literature [35]. For this purpose, the microspheres were subjected to acetoxy mercuration using mercury acetate in acetic acid solution at 120 °C for 3.0 h. Acetoxy mercury groups on the surface of PS-co-DVB microspheres were converted to mercury chloride by interaction with saturated aqueous NaCl solution as described previously [35]. The content of the bromoacetyl groups was calculated as 0.74 mmol per gram microspheres via silver nitrate method as described elsewhere [37,38].

A three necked polymerization system (100 mL) was equipped with a nitrogen gas inlet and reflux condenser. Under a nitrogen atmosphere, CuBr (1.48 mmol, or 0.212 g), H-TETA (1.48 mmol or 0.962 g), GMA (30 mL) and toluene (15 mL) were transferred into the polymerization system. After complexation of copper with H-TETA, PS-co-DVB microspheres (2.0 g) were added to the reaction medium, and the system was closed. Polymerization was carried out at 60 °C for 12 h. At the end of the reaction, the [PS-co-DVB-g-P(GMA)] microspheres were separated from the medium, washed sequentially with toluene (2 × 50 mL) and ethanol (2 × 50 mL) to remove reaction impurities. The P(GMA) grafted microspheres were then dried under reduced pressure at room temperature for 24 h.

Modification of epoxy groups of the P(GMA) fibrous chains into cyclic carbonate groups

Epoxy groups of the P(GMA) chains on the PS-co-DVB microspheres were converted into five-membered cyclic carbonate groups according to a procedure described in the literature [6,39]. For this purpose, 12.5 g P(GMA) grafted microspheres were dispersed in 120 mL dry DMF (*N,N*-dimethylformamide) in the presence of catalytic amount of LiBr (0.445 g, 5.125 mmol) in a 250 mL three necked round bottom flask. It was equipped with carbon dioxide (CO_2) delivery line, calcium chloride guard tube and a reflux condenser. In another vessel, carbon dioxide was generated by drop-wise addition of concentrated sulfuric acid (30 mL, 0.55 mol) on solid Na_2CO_3 (79.5 g, 0.75 mol). The produced CO_2 was directly bubbled into the reaction flask for 2.0 h at 100 °C via a gas delivery line. After saturated the dispersion medium with CO_2,

Please cite this article in press as: G. Bayramoglu, et al., Cyclic-carbonate functionalized polymer brushes on polymeric microspheres: Immobilized laccase for degradation of endocrine disturbing compounds, J. Ind. Eng. Chem. (2017), https://doi.org/10.1016/j.jiec.2017.11.028