Increase in stability of organophosphorus hydrolase by immobilization technique

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INTRODUCTION

The organophosphorous compounds (OPCs) being derivatives of phosphoric or alkylphosphonic acids are widely used as pesticides in agriculture and private life (Costa, 2006). The most toxic chemical warfare agents such as Soman, Sarin and Vx that should be destroyed according to "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction" up to 2012, are also among OPCs of the same group (Efremenko, 2001). The low rates of environmental detoxification of OPCs provoke their accumulation presumably in river and ground waters.

Organophosphorus hydrolase (OPH, EC 3.1.8.1) is capable of hydrolyzing wide variety of OPCs (Efremenko, 2001), thereupon its application in biotechnological decontamination of the compounds is considered to be a basis of advanced bioremediation processes.

The immobilization of enzyme is usually applied to obtain a stable form of OPH allowing its multiple uses in OPCs hydrolysis (Efremenko, 2005b; Efremenko, 2007b). The use of various amino acid sequences genetically introduced to OPH structure as ligands for enzyme immobilization recently became very popular as a tool for biocatalyst preparation (Efremenko, 2005b; Efremenko, 2007a). In general, the use of specific tags of fusion proteins for their immobilization allows combining the isolation, purification and immobilization of the target protein on a carrier in the only step (Efremenko, 2006; Terpe, 2003).

Insertion of polyhistidine sequence containing six or twelve histidine residues at N- or C-terminus of protein molecule was shown to enable combination of effective purification and immobilization of OPH onto metal-chelating carriers. Such immobilization method was established to increase the stability of obtained biocatalysts as compared to soluble forms of OPH derivatives (Efremenko, 2008). It is also known that effective functioning of immobilized biocatalyst in the flow systems depends on a catalytic activity of the enzyme and certain properties of the carrier (its porosity, reusability, sorption capacity in relation to substrates and products, resistance to mechanical deformation, etc.). These aspects were taken into account, when a new immobilized biocatalyst for the treatment of water polluted by OPCs was developed (Efremenko, 2008) using polypeptide with polyHis-tag and OPH-activity (polyHis-OPH) (Efremenko, 2005b). Cryogel of synthetic polymer such as poly(*N,N*-dimethylacryl amide) (cryoPDMAAG) (Lozinsky, 2003) obtained as polymerization product with surface modified by metal-chelating ligands and charged with divalent metal ions was applied as a supermacroporous carrier.

Stability of new immobilized biocatalysts based on the polyHis-OPH and cryoPAAG in the reaction of OPC hydrolysis in the flow systems were investigated in this work.

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MATERIALS AND METHODS

The following chemicals used in the work were purchased from Sigma (St. Louis, MO, USA): O,O-diethyl O-(4-nitrophenyl) phosphate (Paraoxon); imidazole; isopropyl-β-D-thiogalactoside (IPTG); *N,N*-dimethylacrylamide (DMAA); *N,N'*-methylene-bis-acrylamide (MBAA); allyl glycidyl ether (AGE); *N,N,N',N'*-tetramethylethylenediamine (TEMED); cobalt chloride hexahydrate; potassium persulfate. Tryptone and yeast extract were bought from Difco (Detroit, MI, USA). All other chemicals were of analytical grade and purchased from Reachim (Moscow, Russia).
To produce the IDA-cryoPDMAAG, the aqueous solution of DMAA and MBAA mixture was degased with Ar. Then, the AGE was added and the mixture was cooled to 1-2°C. To initiate the reaction of polymerization, the TEMED and potassium persulfate were added. The reaction solution was placed in to the 5-mL syringes, which were frozen at -12°C. After reaction completion the cryogels were modified by iminodiacetic acid and charged with Cu^{2+}-ions. The immobilization of polyHis-tagged OPH was performed accordingly to the previously elaborated procedure (Efremenko, 2008).
The investigation of the catalytic characteristics of biocatalyst based on immobilized polyHis-tagged OPH was carried out as it was previously reported (Efremenko, 2005b; Efremenko, 2008). The accumulation of 4-nitrophenolate anion (p-NP) as a product of enzymatic hydrolysis was monitored (25°C, 405 nm) using 8300 Uvicord II spectroscopy system (Pharmacia, Sweden). Aqueous solution of Paraoxon (15 mM) was used as a stock solution. In all cases the catalytic reaction was initiated by pumping of 1 mM substrate solution in the 100 mM Na-carbonate buffer (pH 10.5) through the column filled by immobilized biocatalyst at flow rate of 60 mL/h. One unit of enzymatic activity was defined as the quantity of the enzyme necessary to hydrolyze 1 μmol of substrate per 1 min at 25°C. Immobilized biocatalysts washed by 4-5 volumes of buffer in the flow system at the rate of 60 mL/h were stored at +8°C between the uses.

RESULTS AND DISCUSSION

The operational and storage stabilities of immobilized preparations were investigated. The residual activity of immobilized biocatalysts was controlled after a long-term usage in the flow system for hydrolysis of pesticide Paraoxon under continuous (**Figure 1**) and batch (**Figure 2-4**) conditions.

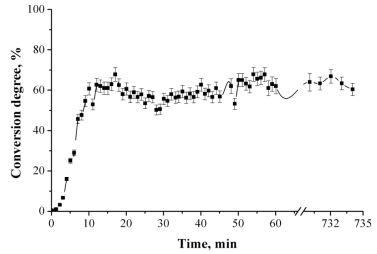

Figure 1: Conversion degree of 1 mM Paraoxon by immobilized biocatalyst (4.5 mL) prepared on the basis of His_6-OPH and used in the flow system under continuous conditions

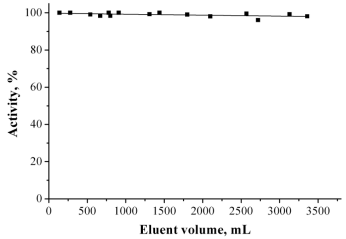

Figure 2: Operational stability of immobilized enzyme preparation (4.5 mL) in the flow system

Immobilized preparation obtained on the basis of His_6-OPH and Cu-IDA-cryoPDMAAG possessed a high enough operational stability under continuous conditions for, at least, 12 h (**Figure 1**). This biocatalyst was capable of hydrolyzing 1 mM Paraoxon in the flow system, providing almost constant conversion degree of substrate equal to 60-65%. In the experiments the total volume of substrate solution passed through the column with immobilized biocatalyst was over 150 volumes of biocatalyst. Under such conditions the biocatalyst retained up to 100% of initial activity. The sample of immobilized enzyme was not washed among its working cycles, and the residual concentrations of hydrolysis products were not removed from the biocatalyst. Product inhibition is a well known phenomenon for soluble OPH (Donarski, 1989), but it was not observed in the case of immobilized enzyme.

The trial of the immobilized biocatalyst for a long time under flow conditions (more than 700 column volumes) didn't lead to elution of His_6-OPH immobilized onto Cu-IDA-cryoPDMAAG from the carrier (**Figure 2**).

100 mM Na-carbonate buffer (pH 10.5) was appeared to be the most appropriate medium for the storage and application of the immobilized biocatalyst (**Figure 3**). The use of 50 mM PBS (pH 7.5) as storage buffer led to the 50% activity decrease during the first 20 days, and the residual activity was stable for further trials (**Figure 4**). Substitution of PBS for the Na-carbonate buffer (pH 10.5) enabled to restore 20% of initial activity of inactivated immobilized biocatalyst.

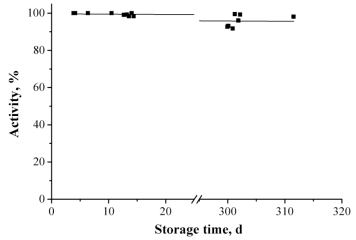

Figure 3: Residual activity of immobilized His_6-OPH (4.5 mL) determined periodically in the flow system after its storage in the 100 mM Na-carbonate buffer (pH 10.5)

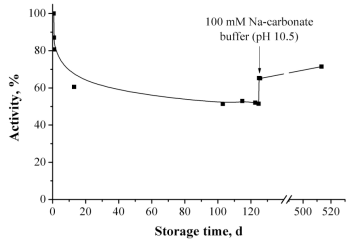

Figure 4: Residual activity of immobilized His_6-OPH (4.5 mL) determined periodically in the flow system after its storage in the 50 mM PBS (pH 7.5)

CONCLUSIONS

Thus, the stability of developed immobilized biocatalyst under various conditions was studied. Factors affecting retention of catalytic activity of the immobilized biocatalyst for its long-term storage and periodical usage were investigated. In comparison with soluble form of His_6-OPH the stability of immobilized biocatalysts was significantly increased. As a result, catalytic activity of immobilized enzyme retained stable, at least, for 1 year (**Figure 3-4**).

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