P-044 **Enzyme immobilization for destruction of pesticides in soils**

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INTRODUCTION AND OBJECTIVES

Organophosphorus compounds (OPCs) are widely used as pesticides in agriculture. That leads to their accumulation in soils and then in agricultural goods composing a threat to human health.

Organophosphorous hydrolase (OPH, EC 3.1.8.1), containing six residues of histidine on N-terminus of protein molecule (His_6-OPH), hydrolyses organophosphorus pesticides and warfare agents (Efremenko 2001). That provides expediency of using this enzyme for OPC destruction and soil bioremediation.

The development of inexpensive and effective biocatalyst based on immobilized His_6-OPH for destruction of OPCs in soils was the main task of the work. The carrier for immobilization should meet some important requirements: preservation of high and stable enzymatic activity, ecological acceptability, low cost and simple immobilization procedure. So, the screening of mostly appropriate carrier for His_6-OPH immobilization was undertaken.

MATERIALS AND METHODS

Among carriers taken for immobilization of His_6-OPH were straw, sawdust, activated coal Hydraffin CC (Donay Carbous), sibunit (The G.K.Boreskov Institute of catalysis, Russia), diatomaceous earth, vermiculite (Diatomit, Russia), sand.

His_6-OPH was used as a non-purified preparation, obtained after desintegration by sonifier of *E.coli* SG13009[pREP4] cells (Efremenko 2005)

Immobilization of His_6-OPH was carried out by adsorption on different carriers to compare their immobilizing capability. 2 ml of enzyme (300 Unit/ml) was added to 0.5 g of the above mentioned carrier and soaked for 2-4 hours at 22°C with gentle agitation. After immobilization the material was washed several times with buffer to remove non-immobilized enzyme.

The enzymatic activity was measured with the substrate Paraoxon. The enzymatic activity was measured spectrophotometrically by monitoring the accumulation of p-nitrophenol (the hydrolytic product of Paraoxon) at 405 nm.

RESULTS AND DISCUSSION

The screening of different carriers for the immobilization of His_6-OPH showed that the maximum of enzymatic activity was established for straw as carrier (Fig. 1). It was confirmed by literary analysis that wastes of wood-working industry (Mahmoud 2007), specifically, sawdust and wastes of agriculture, for example straw, can act not only as ecologically acceptable and cheap carriers for enzymatic immobilization, but also as soil structurators.

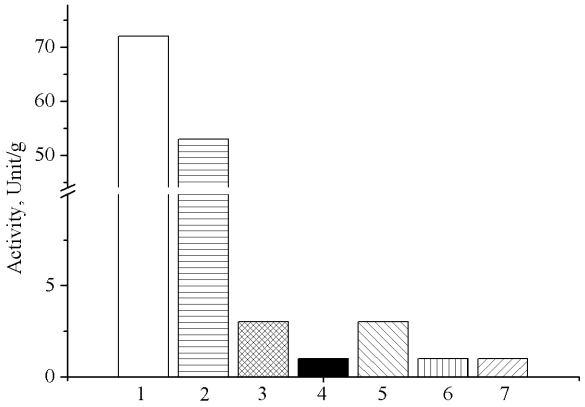

Figure 1: Activity of immobilized His_6-OPH on: 1 – straw, 2 – sawdust, 3 – vermiculite, 4 – activated coal, 5 – sibunit, 6 – sand, 7 - diatomaceous earth

It was established that cellulose-containing carriers are the best choice for immobilization of His_6-OPH according to much higher level of activity as compared to other carriers. Possible explanation of the result is the presence of pores in cellulose-containing carriers with sizes corresponding to protein size. Besides there are numerous noncovalent interactions between His_6-OPH and the surface of straw and sawdust.

For more detailed investigations sawdust of deciduous (oak - I, birch - II) and coniferous (fir-III) trees were used as carriers for immobilization of His_6-OPH as far as they have different densities and structure (pectinaceous and albuminous networks, binding glycans)

According to results (Tab. 1), immobilized biocatalyst (IBC) obtained on the basis of coniferous sawdust (III) possessed the highest activity. Its activity was in 2.2-3.5 times higher than activity of IBC on the basis of deciduous sawdust. The washing of IBC with the buffer (10 мМ $NaHPO_4$, 5 мМ $NaHCO_3$, pH 7.5) in the flow reactor simulating rainfalls (flow rate 3.8 mm/ch), allowed to establish decrease in initial activity of IBC with the subsequent establishment of the parameter at constant level.

It appeared that IBC prepared on the basis of deciduous sawdust had the highest activity at equal protein concentrations immobilized on carriers. It was obvious that in the case of such carriers the efficacy of immobilization process depends on densities of used sawdust. Whereas density of sawdust of coniferous trees practically was 1.5 times lower than the same characteristic known for samples with deciduous trees (Table 1). Obviously, high enough absorption of the protein was revealed for these carriers.

Table 1: Characteristics of IBC based on different types of sawdust and enzyme His_6-OPH.

Sawdust	Average density, kg/m^3	Initial activity of IBC, U/g	Activity of IBC after washining, U/g
I	690	141	53.5
II	650	88	50.0
III	450	308	20.0

The measurement of buffer volume (10 мМ $NaHPO_4$, 5 мМ $NaHCO_3$, pH 7.5), which is necessary to wash IBC based on different carriers until establishment of constant concentration of enzyme in the biocatalyst, was extremely important. This indicator can be used further for an estimation of IBC activity in soils in the presence of rainfalls or agricultural waterings. There was no decrease in quantity of immobilized enzyme after washing of 0.2 g of IBC with 25 ml of buffer (Fig. 2).

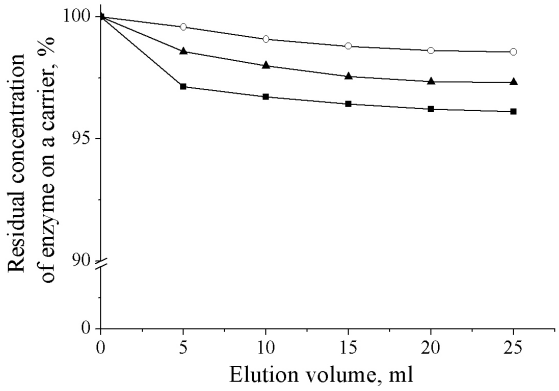

Figure 2: Residual concentration of immobilized emzyme in the frame of carriers: I (▲), II (○), III (■), after their washing with various volumes of buffer.

It is obvious that there are various average norms of rainfall in different climatic zones : from 35 mm/month (for the Orenburg region) to 85 mm/month (for Moscow). The analysis of rates of sample washing conducted in experiments obviously showed that they are comparable with natural. On the basis of obtained results it was concluded that there is slight elution of enzyme from a carrier in soil and losses of activity didl not connect with that.

Immobilization of His_6-OPH on sawdust with different levels of delignification showed that maximum activity of IBC corresponded to the highest level of delignification (Fig. 3). More developed surface was generated during delignification process of cellulose-containing raw materials. That led to increase in quantity of His_6-OPH conacting with the carrier and increase in activity of the obtained biocatalyst.

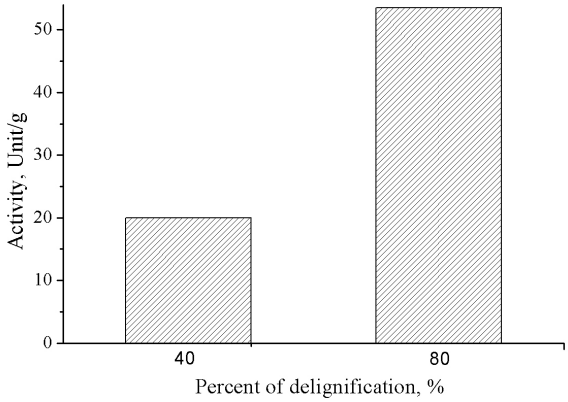

Figure 3: Activity of IBC according to delignification level.

Delignification of cellulose-containing materials led to increase in adhesion possibility of proteins (Mahmoud 2007). This fact was also confirmed by the highest activity of IBC obtained with straw as a carrier (Fig. 1) containing lower amount of lignin than sawdust.

CONCLUSIONS

Screening of cheap suitable carriers for immobilization of His_6-OPH for soil remediation shows that delignificated cellulose-containing carriers (straw, sawdust) possess the best characteristics. Moreover, obtained results could be used for the development of a bioremediation strategy of soils polluted by OPCs.

ACKNOWLEDGEMENT

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