WASTE-WOOD DERIVED BIOCHAR AS A SUPPORT FOR HORSERADISH PEROXIDASE IMMOBILIZATION

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Abstract
In this paper, the suitability of waste-wood derived biochar particles as a support for the horseradish peroxidase (HRP) immobilization by adsorption method was investigated. The change in enzymatic activity of the immobilized enzyme at different values of pH and temperature, as well as stability over time, was measured. The results showed that HRP can efficiently bind to biochar particles by adsorption. The immobilized enzyme shows high activity (>80%) at a wide range of pH (7-9) and temperature (20-50°C). The immobilized enzyme retains 22% and 40% of its activity during storage at temperatures of 25 and 10°C after a period of 30 days, respectively.

Introduction
Peroxidases represent a large group of enzymes that have been used for environmental remediation purposes [1]. However, the application of enzymes in industry has certain disadvantages due to their instability during time and non-reusability, which could be avoided by binding the enzyme on a suitable solid support. Biochar is an attractive alternative material for enzyme immobilization due to its low cost and readily available starting materials. Also, due to its porous carbon nature and large surface area these materials are very useful as a support for enzyme immobilization [2]. The aim of this work was to investigate the suitability of waste-wood derived biochar particles as a support for the HRP immobilization by adsorption method.

Experimental
Wood biochar (BC) was obtained from sawdust of beech and oak wood mixture by pirolysis at 700°C under atmospheric pressure (Basna doo, Čačak, Serbia). In order to introduce the oxidative functional groups on the surface of BC, their functionalization with concentrated nitric acid was performed according to the method given in Naghdi et al [3]. HRP was extracted from horseradish root and immobilized onto functionalized BC by adsorption method. The enzyme activity was measured according to Worthington method [4]. Surface morphology characteristics of BC and immobilized HRP onto BC were investigated by using scanning electron microscopy (SEM) JEOL JSM-6460LV on 25 kV. The impact of different temperature (30–80°C) on enzyme activity was measured at constant pH7, and impact of different pH (4-9) on enzyme activity was measured at ambient temperature. The storage stability of immobilized HRP was evaluated by measuring their peroxidase activity during one month period. Results for enzyme activity were presented as relative values (%).

Results and discussion
Enzyme immobilization by adsorption (with hydrophobic interactions) provides binding of the enzyme onto the surface of the support. The activity of immobilized HRP onto BC was 11.2±1.4 U/g BC at pH 7.
The surface morphology of the following samples: a) BC, b) functionalized BC and c) functionalized BC after enzyme immobilization, are present in Figure 1. All samples are

characterized by a heterogeneous surface and a developed porous structure. The pore size is in range 7.81-13.0 μm. After treatment with nitric acid during the functionalization process, no visible changes on the surface of BC were observed (Figure 1b). The size of HRP is only 2-4 nm, so it can easily be incorporated into the pores of BC (Figure 1c).

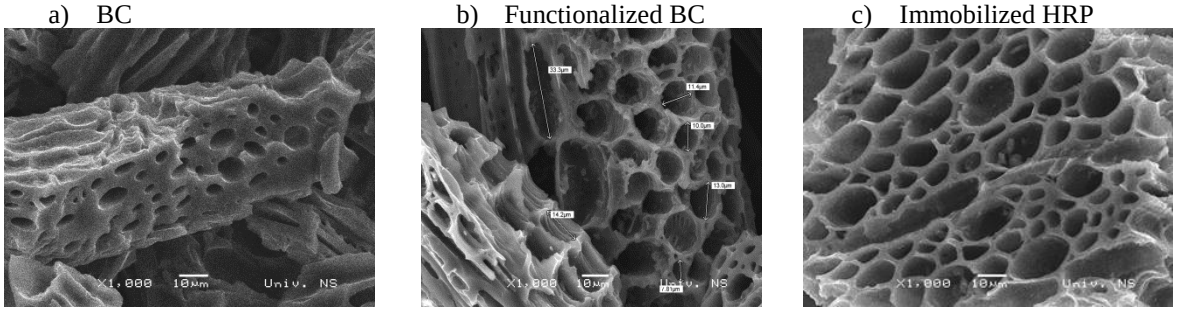

Figure 1. Scanning electron micrographs of a) non-functionalized biochar, b) functionalized biochar and c) HRP immobilized onto functionalized biochar

The activities of HRP immobilized onto functionalized BC at different a) temperatures and b) pH values are present in Figure 2. It could be seen that the activity of investigated enzyme equally depends of both factors. The immobilized enzyme shows high activity (> 80%) at temperatures 20-50°C, while further increasing in temperature decreases the enzyme activity due to their denaturation. On the other hand, the immobilized enzyme is more active in neutral and base than acid conditions, with the highest activity recorded at pH 9.

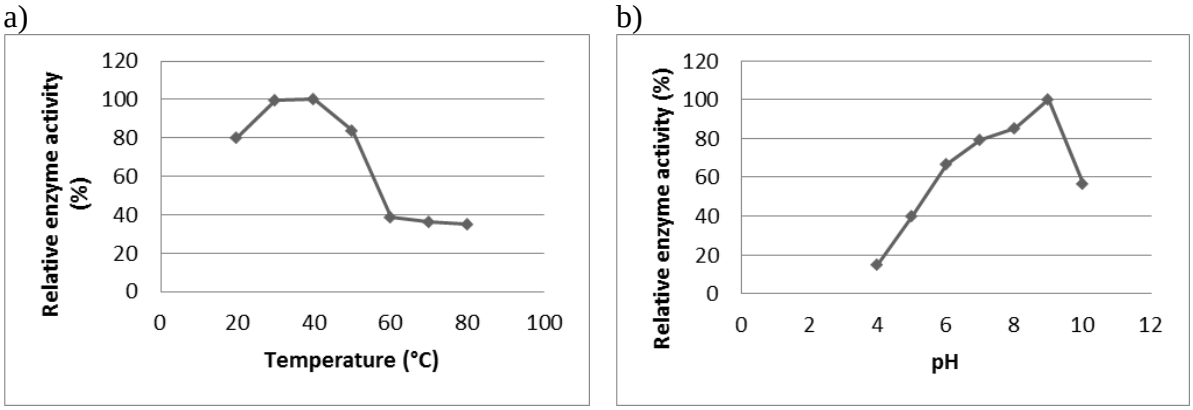

Figure 2. The impact of a) temperature and b) pH values on activity of immobilized HRP

The change in enzyme activity over a period of one month at 10 and 25°C is shown in the Figure 3. The HRP immobilized onto functionalized BC retains around 80% of its activity in the first 5 days at both temperatures. After a period of one month the activity decreases up to 40% and 22% during storage at temperatures 10°C and 25°C, respectively.

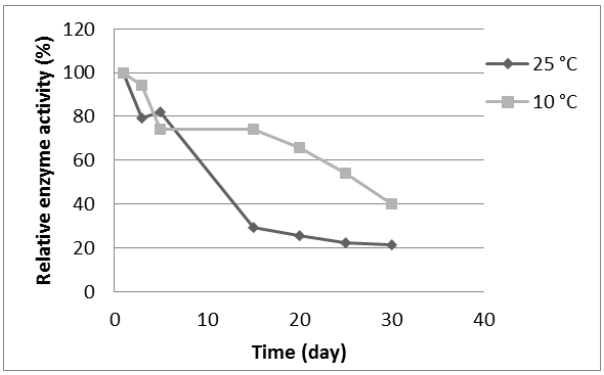

Figure 3. The stability of immobilized HRP during time

Other researchers come to the same conclusion that the immobilized HRP exhibits higher thermal stability, pH stability, storage stability than the free HRP [2,5].

Conclusion

Immobilization of HRP onto BC via hydrophilic interactions yields an enzyme with high activity, stable to various variations of pH and temperature. The immobilized enzyme remains stable for a longer period if stored at a temperature of 10°C. Hence, biochar is proved as a carbon material with a great potential to improve enzyme immobilization efficiency.

Acknowledgements

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