



*J. Serb. Chem. Soc.* 83 (5) 549–559 (2018)  
JSCS–5095

Journal of  
the Serbian  
Chemical Society

JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

UDC 542.92.000.57+547.591:504.53.054+  
547.681+547.814

*Original scientific paper*

## Degradation of polycyclic aromatic hydrocarbons in contaminated soil by immobilized laccase

XIN WANG, SHI YU SUN, ZI JUN NI, ZHAO XING LI and JIA BAO\*

*School of Science, Shenyang University of Technology, Shenyang 110870, China*

(Received 4 October 2017, revised 15 January, accepted 22 January 2018)

**Abstract:** The biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soils by laccase is reported. However, the low laccase activities of free laccase have limited its applications in environmental bioremediation. In this study, polluted soil was made with 3:1 volume ratio of soil to PAH solution. Subsequently, the adsorption–cross-link composite immobilization method was applied to immobilize laccase derived from fungi onto nylon net and chitosan, respectively. These two kinds of immobilized laccase were used in the degradation of pyrene (Pyr) and benzo[*a*]pyrene (BaP), and their degradation efficiencies under different temperature and pH conditions were investigated. Consequently, the optimal laboratory experimental parameters were determined as follows: first, compared with free laccase, the degradation rates of Pyr and BaP by immobilized laccase increased by around 10–30 %; second, the degradation efficiency of chitosan as a carrier of immobilized laccase was much better than that of nylon net as a carrier of the immobilized laccase; finally, when the temperature was set at 40 °C and the pH was set at 4, the degradation efficiency achieved by immobilized laccase was the best.

**Keywords:** immobilized laccase; polycyclic aromatic hydrocarbons (PAHs); soil remediation; nylon net; chitosan.

### INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbons that contain more than two benzene rings,<sup>1</sup> including over 150 kinds of compounds, such as naphthalene, anthracene, phenanthrene and pyrene. They are considered toxic chemicals that are mainly formed during the incomplete combustion of coal, crude oil, gas, or other organic substances. Numerous studies<sup>2,3</sup> have shown that most of the low molecular weight PAHs have one, two, or three aromatic rings, and they are acutely toxic. Most of the high molecular weight PAHs have more than three aromatic rings, which are highly mutagenic, teratogenic, and carcinogenic to

\* Corresponding author. E-mail: baojia@sut.edu.cn  
<https://doi.org/10.2298/JSC171004022W>

humans and animals. Some studies have reported a potential relationship between the death from lung cancer of humans and PAHs.<sup>4</sup> For instance, in a study conducted in the Henan province, Feng *et al.*<sup>5</sup> revealed that the accumulation of PAHs is the highest in roots and the lowest in grains, and that the consumption of wheat grain caused potential risk of cancer. Some researchers found that the incidence of lung cancer was also closely related to air pollution by benzo[*a*]-pyrene.<sup>6</sup> In addition, Jung *et al.*<sup>7</sup> maintained that exposure to PAHs in the air around New York City, especially in the warmer seasons, could increase the risk of cancer and mutations. The common sources of PAHs found in the environment are both natural and anthropogenic. Natural sources include forest and pasture fires, oil leakages, volcanic eruptions and plant secretions. Anthropogenic sources of PAHs include the burning of fossil fuel, coal tar, wood and garbage, municipal solid waste incineration, petroleum spills and used lubrication oil.<sup>8,9</sup> Hitherto, PAHs were considered to be everywhere and pollute air, soil, or water, and their migration pathways into the environment include volatilization, photo-oxidation, chemical oxidation, adsorption on soil particles, leaching and microbial degradation. PAHs are resistant pollutants. Generally, the degradation rate of PAHs are reduced with increasing molecular weight.<sup>10</sup> Therefore, under natural conditions, compared with high molecular weight PAHs, low molecular weight PAHs could be degraded rapidly. Furthermore, with increasing molecular weight, the toxicity and durability of PAHs will also increase. Due to their high toxicity and environmental persistence, PAHs are classified as carcinogens in many organizations, including the United States Environmental Protection Agency (USEPA), the International Agency for Research on Cancer (IARC), and the National Occupational Safety and Health Administration (OSHA), *etc.* Resulting from these classifications, safety issues related to the degradation of these compounds have been attracting more and more research interests.<sup>11–13</sup>

Laccase (EC 1.10.3.2), also named phenolase, is a kind of Cu-containing polyphenol oxidase. It is a protein in *Rhus vernicifera* that can catalyze the immobilization of coatings and was first identified in 1883 by Yoshida, a famous Japanese scholar.<sup>14</sup> More than a decade later, Keshavarzifard *et al.*<sup>15</sup> also found the enzyme in fungi and named it 'laccase'. Laccase is generally divided into plant laccase, fungal laccase, and bacterial laccase. At present, most of current research is based upon fungal laccase, with white-rot fungus being the most important producer of fungal laccase. The relative molecular masses of fungal laccase are between 50 and 130 kDa, and its isoelectric points are from three to six. Laccase has strong stability and broad substrate specificity. Polyphenols (a structural class of mainly natural, but also synthetic or semisynthetic, organic chemicals characterized by the presence of large multiples of phenol structural units), aromatic amines, benzyl mercaptan, and methoxybenzene can be used as the object of laccase oxidation. Compared with the direct degradation of pol-

lutants by microorganisms, enzyme-catalyzed reactions have high decomposition efficiencies, low toxicity, a wide application range, and simple operation. Simultaneously with the development of modern biological technology, it has also become possible to obtain large amounts of cheap laccase by effective separation, purification, gene expression, and recombinant technology. In recent years, due to its abilities to degrade phenolic compounds, laccase has been widely applied to different biotechnological processes. Laccase from fungus has also been recognized as a potential candidate material for the bioremediation of PAHs.<sup>16–18</sup>

Over the past decade, immobilized enzyme technology has rapidly developed, with more and more new carriers applied for the immobilization of various enzymes.<sup>19–21</sup> New carrier materials based on immobilized laccase could increase the stability and repetition for the utilization of laccase, and reduce the cost of its degradation.<sup>15</sup> Immobilized laccase has been mainly used in the production of food, pharmaceuticals, and other biological degradation techniques.<sup>22–24</sup>

In this study, two methods using immobilized laccase were applied to the remediation of PAHs in soils, under different temperature and pH conditions. The objectives of this study were: *i*) to screen optimal carriers for the immobilization of laccase, which should be cheap and readily available, and widely applied, and *ii*) to effectively improve the activity of laccase by choosing suitable immobilization methods, and then provide a new way for the degradation of PAHs in organically polluted soils using immobilized laccase.

## EXPERIMENTAL

### *Materials and methods*

Fungal laccase was purchased from the Beijing Institute of Microbiology of the Chinese Academy of Sciences. The soil was collected from non-polluted surface soil (0–20 cm depth) at the Ecological Station of the Chinese Academy of Sciences. Nylon mesh (100 mesh), chitosan (deacetylation degree 90.3 %), glutaraldehyde and other reagents were purchased from the Tianjin Bo Di Chemical Co., Ltd. (China). A UV721 UV–Vis spectrophotometer from the Shanghai Precision Science Instrument Co., Ltd was used. An HP 1090-II liquid chromatograph and a diode array detector (DAD) were used for the determination of the degradation rate of the PAHs.

### *Methods*

*Preparation of crude laccase.* A certain quantity of straws (each about 3 cm long) was weighed into a conical flask and then water was added and the straws soaked for one day. The wet straws were sterilized at a high temperature and high pressure for 30 min. After cooling, the straws were used as the raw material to produce the enzyme culture medium. White-rot fungi (*Lenzites betulinus*) were grafted into this culture medium and fostered in an incubator at 26 °C, then 100 mL aseptic liquid was added and soaked at 26 °C and 120 rpm agitation for 24 h. To remove the impurities, the mixture was centrifuged for 15 min at 4000 rpm and the supernatant was collected as crude laccase.

*Immobilization procedure.* Two kinds of carriers were used to immobilize laccase and the adsorption–cross-link method was adopted to immobilize laccase. The cross-linking agent was glutaraldehyde.

Nylon net, a hydrophilic carrier, was the adopted carrier material, and linked by amide bonds. Nylon net has the advantage of low cost, stable chemical properties and good operability. The nylon net was cut into small pieces (1 cm×1 cm), and then soaked in formaldehyde solution with CaCl<sub>2</sub> (19 %) and H<sub>2</sub>O (19 %) for 15 min. The nylon net was placed into HCl (5 mol L<sup>-1</sup>) for 40 min, after which the sample was washed and dehydrated, and then the pH of the nylon net was adjusted to neutral before repeated dehydration. Then, the nylon net was immersed in a glutaraldehyde solution (5 %) for 6 h, placed in 30 units of laccase for 8 h and then flushed with phosphate buffer to reduce the pH to 7. The laccase recovery rate was 49 % under these conditions.

The other carrier was chitosan. Chitosan was obtained by deacetylation of chitin, which is rich in free amino groups. Chitosan is an admirable carrier for laccase immobilization with many advantages, such as abundant resources, good biocompatibility, good hydrophilicity and resistance to microbial decomposition.<sup>25</sup> Specific operational steps in detail were as follows: chitosan was dissolved in glacial acetic acid until chitosan precipitation. NaOH (2 mol L<sup>-1</sup>) was taken into the glacial acetic acid, and the pH of the chitosan solution was adjusted to neutral before dehydration. Carrier (0.1 g) was accurately weighed and added to a glutaraldehyde solution (15 mL, 5 %) for eight hours, and subsequently 30 units of laccase was added to the glutaraldehyde solution. After 12 h, the immobilized carrier was cleaned with phosphate buffer to reduce the pH to 7. Under these conditions, the enzyme recovery rate was 50 %.

*Determination of free laccase activity.* 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, was used to determine the activity of free and immobilized laccase. The reaction solution (1 mL) was made of HOAc-NaOAc (500 μL, 50 mM, pH 4), H<sub>2</sub>O (390 μL), ABTS (100 μL, 500 μM), and laccase liquid (10 μL). The reaction solution was placed in the UV-Vis spectrophotometer and the temperature was set as 28 °C. Then the increase in absorbance during 3 min at 420 nm was used to calculate the free laccase activity ( $\epsilon_{420} = 36000$  L/(mol cm)). The free laccase activity was calculated by the following formula:

$$U_l = \frac{1000\Delta AV}{\Delta t \epsilon_{420} V_0 l}, \quad U_s = \frac{U_l V_1}{m_s}$$

The culture condition of the first one in liquid, the second one in solid, when the condition was solid culture, the unit of activity of laccase enzyme was U/g, in which the  $V_1$  was the sterile extraction solution added during extraction,  $m_s$  was the quality of the added straw culture medium.  $\Delta A$  was increment of absorbance during three minutes,  $V$  was reaction solution (1 mL),  $\Delta t$  was 3 min,  $V_0$  was laccase liquid (0.01 mL).  $l$  was the inner diameter of cuvette,  $l = 1$  cm, and  $m_s$  was the quality of the medium. One unit (U) of laccase activity was defined as the amount of laccase used for catalytic oxidation of ABTS (1 μM) after 1 min.

*Determination of immobilized laccase activity.* Immobilized laccase (0.01 g) was accurately weighted and added into beaker, reaction solution (1.98 mL) was made of HOAc-NaOAc (1000 μL, 50 mM, pH 4), H<sub>2</sub>O (780 μL), ABTS (200 μL, 500 μM), and reaction solution was mixed for 10 min at room temperature. Next, the supernatant was put into the UV-Vis spectrophotometer and the reaction started. A free laccase activity was determined as an increase in the absorbance at 420 nm during 3 min. Measurement data was used to calculate the immobilized laccase activity. The activity was calculated by the following formula:

$$U_i = \frac{1000\Delta AV}{\Delta t \epsilon_{420} M_0}$$

$U_i$  was the activity of immobilized laccase  $V$  was 2 ml of reaction solution and  $M_0$  was 0.01 g immobilized laccase.

*Recovery of enzyme activity of immobilized enzyme.* When laccase was immobilized, the stability of laccase would be improved obviously, but the enzyme activity would have some loss. The inactivation of laccase could be expressed by relative enzyme activity, that is, the recovery rate of immobilized enzyme activity. The specific calculation formula was as follows:

$$A = \frac{100U_i}{U_f}$$

In the formula,  $A$  was the recovery rate of enzyme activity,  $U_i$  was the total activity of immobilized enzyme and  $U_f$  was the total activity of the added free enzyme.

*Preparation of contaminated soil.* An amount of 0.1 g of pollutants, pyrene (Pyr) and benzo[a]pyrene (BaP), was separately weighed and added into 500 mL volumetric flask containing methanol. Then, it was fully dissolved in the ultrasonic bath and constant volume. When a 15 mL sample was added into 250 mL Erlenmeyer flask containing 30 g of soil, the pollutant concentration was 100  $\mu\text{g/g}$ . After the soil was dried naturally, 90 mL seed medium was added to the triangle bottle to make mud, then the ratio of soil to water was 3:1.

*Methods for analysis and determination of soil samples.* The soil samples were centrifuged, freeze-dried and sieved, then packed in bags and placed in freezers. 1 g soil sample and 20 mL of dichloromethane extract liquid was taken into 100 mL glass centrifuge tube, and then oscillated continuously for 2 h (changing water to keep water temperature not more than 35 °C). After centrifugation for 5 min with 3500 rpm, the upper liquid was taken out and constant volume with dichloromethane to 20 mL. 1 mL liquid was filtered by silica gel column, then eluted with *n*-hexane and dichloromethane (1:1), dried with nitrogen and constant volume with methanol to 1 mL to be tested.

*Determination of the residue.* The collected water samples and 10 mL dichloromethane were transferred into 100-mL separating funnels, shaken for 5 min and left to stand for 3 min. The lower organic phase was transferred into a KD concentrator and the process was repeated a further two times. Then, the organic phase was concentrated to 1 mL at a temperature of 55 °C, dried with nitrogen, made up to 1 mL with methanol and finally transferred to a liquid chromatographic sample bottle for measurement.

*Experimental design.* The laccase immobilized by the two methods was used to repair soil contaminated by PAHs. Mud and soil were in volume ratio of 3:1, the solution was HOAc–NaOAc buffer (pH 4), and the initial concentration of pollutants was 100  $\text{g mL}^{-1}$ . The immobilized laccase added into the soil was 1 g per 50 mL, and free laccase was used as the control. Some of the environmental factors encountered in the experiment need to be studied further. The first one was different temperatures, *i.e.*, 20 °C (room temperature) and 40 °C, with the pH set to 4. Secondly, the pH value was set at 4 or 6, with the temperature at 40 °C. The running time of an experiment was 72 h.

#### HPLC conditions

The mobile phase was prepared with methanol and distilled water. An alkyl silica gel column was used. The detection UV wavelength was set to 240 nm for Pyr and 290 nm for BaP. The column temperature was 40 °C and the flow rate was 0.800  $\text{mL min}^{-1}$ .

### Data processing

Microsoft Excel software was applied to process all the experimental data. SPSS 17.0 was applied for the statistical analysis, and  $p < 0.05$  was considered as a significant difference.

## RESULTS AND DISCUSSION

### The recovery rates of immobilized laccase and free laccase

As shown in Fig. 1 in the case of different environmental conditions or different carriers, the recovery rates of laccase had obvious differences. From the point of recovery rates, immobilized laccase had greater activity than free lac-

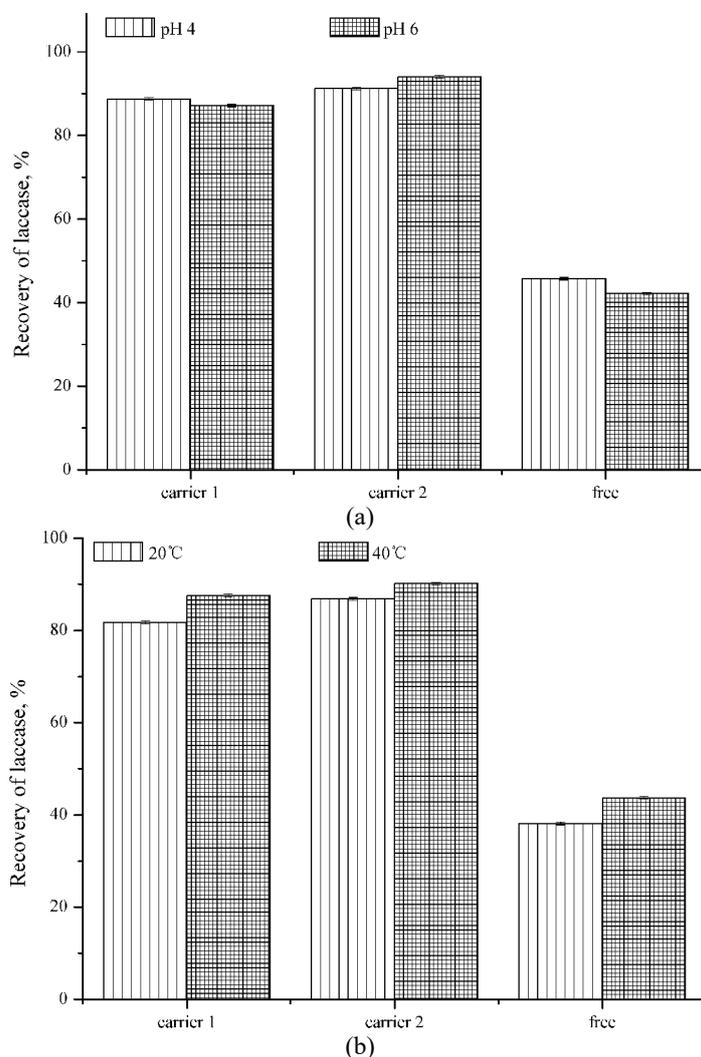


Fig. 1. The recovery of the immobilized laccase and free laccase under different conditions: pH (a) and temperature (b). Carrier 1 was nylon net; carrier 2 was chitosan.

case, in addition, compared with nylon net, the laccase was immobilized on chitosan had higher degradation rates of Pyr and BaP. However, the laccase lost about 50 % of activity during the immobilization process.

With the constant development of immobilized technology and the deepening of the study of laccase, the application of immobilized laccase has demonstrated a positive contribution to the literature. At present, immobilized laccase has been employed in areas of the decolorization of dyes,<sup>26,27</sup> degradation of various pollutants,<sup>19,28</sup> and biosensing.<sup>25,29</sup> Hitherto, most of the studies that have been performed on immobilized laccase were aimed at the degradation of organic contaminants in water.<sup>26,27,30</sup> In this study, fungal laccase was immobilized on nylon net and chitosan, and then the immobilized laccase was applied to the degradation of pyrene (Pyr) and benzopyrene (BaP) in contaminated soil. The results indicated that the methods adopted in this study were effective. In addition, laccase has been successfully immobilized on many different materials, such as silica gel,<sup>31</sup> non-porous acrylate beads<sup>27</sup> and magnetic chelator particles.<sup>32</sup> In the present study, nylon net and chitosan were used as immobilization carriers and have advantages of being cheap, practical and readily available. This could reduce the cost of the application and simplify the operational processes of immobilized laccase. Previous studies<sup>33</sup> have shown that through immobilization, the loss of laccase activity decreased greatly, and the activity of laccase was generally considered stable. Under different pH values, it could be seen that the laccase can maintain a certain activity in near neutral or acid conditions.

#### *The influence of pH on the immobilized laccase degradation of Pyr and BaP*

After immobilization, the two kinds of immobilized laccase were used to repair PAHs-contaminated soil. In this part, the pH value was set at 4 or 6, with the temperature at 40 °C. The running time of the experiment was 72 h. As shown in Figs. 2 and 3, when the temperature was set at 40 °C, both immobilized laccase and free laccase could effectively degrade Pyr and BaP under different pH during 72 h. The degradation rate of Pyr exceeded 80 % (Fig. 2), and the degradation rate of BaP exceeded 40 % (Fig. 3). Compared with pH 6, two kinds of immobilized laccase had higher degradation rates of the pollutants at pH 4. According to the statistical analysis, the two kinds of immobilized laccase had significantly different degradation effects on Pyr and BaP at different pH values. The experimental results were similar to those obtained in other studies.<sup>34</sup>

#### *The influence of temperature on immobilized laccase degradation of Pyr and BaP*

When the pH was set at 4, the two kinds of immobilized laccase had different degradation effects on Pyr and BaP under different temperatures (Fig. 4). At 20 or 40 °C, both the degradation rates of Pyr and BaP exceeded 80 and 50 %, and it could also be seen that the immobilized laccase had a better degradation

effect than free laccase. Zafra *et al.*<sup>35</sup> found that the optimum temperature range was 15–20 °C for immobilized laccase, and that laccase was easy to inactivate at high temperatures.

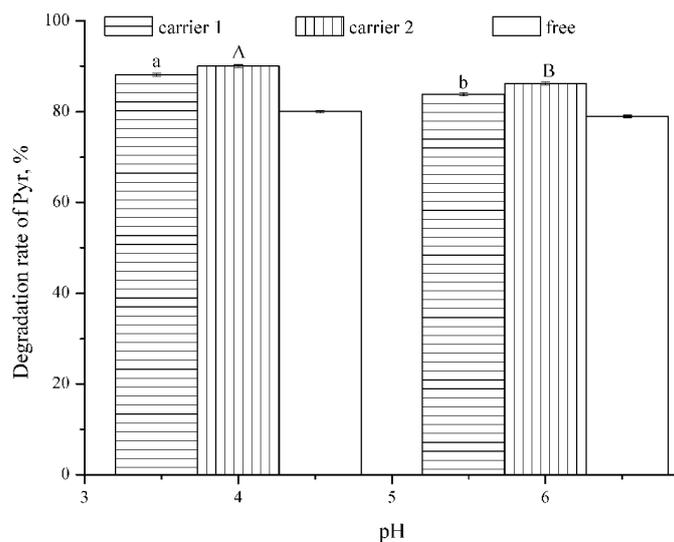


Fig. 2. The influence of pH on the ability of the immobilized and free laccase to degrade Pyr; different letters indicate significant difference in the degradation rate at different pH values ( $p < 0.05$ ).

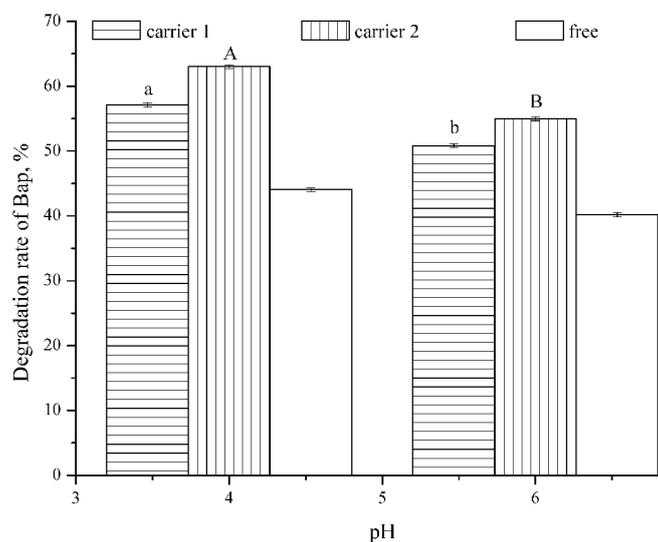


Fig. 3. The influence of pH on the ability of the immobilized and free laccase to degrade BaP; different letters indicate significant difference in the degradation rate at different pH values ( $p < 0.05$ ).

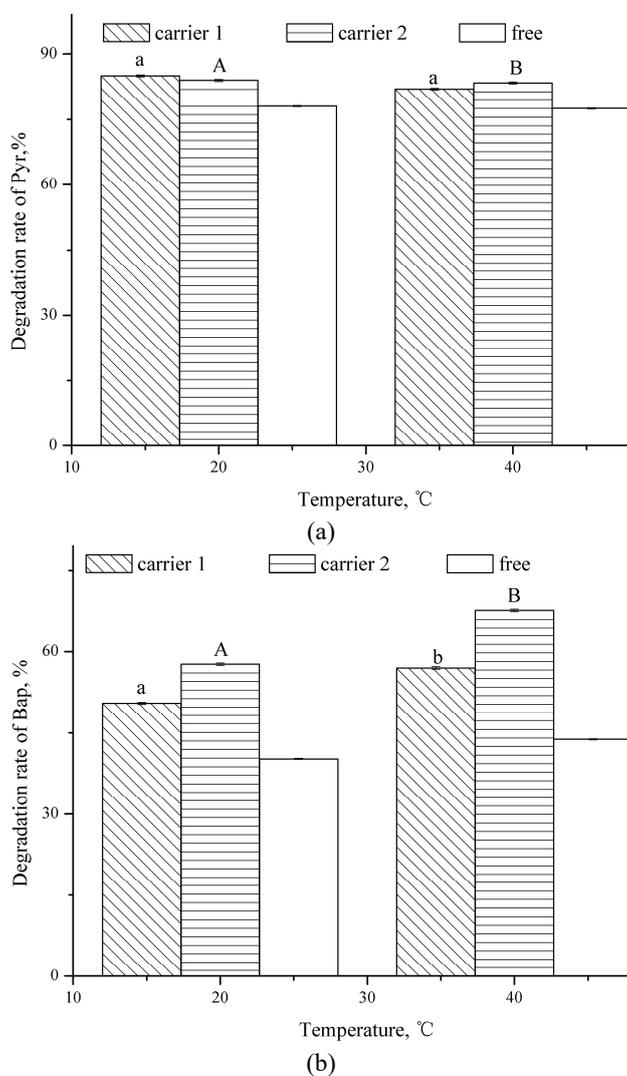


Fig. 4. The influence of temperature on the ability of the immobilized and free laccase to degrade Pyr (a) and BaP (b); the different letters indicate significant difference in the degradation rate at different temperatures ( $p < 0.05$ ).

#### CONCLUSIONS

In summary, according to the present study, it could be seen that immobilized laccase and free laccase had a great degradation capacity for Pyr and BaP. When the temperature was set at 20 or 40 °C and pH was set at 4 or 6, the degradation of immobilized laccase was improved. Moreover, several factors should be considered in the application process. First, considering the time of remediation, if a short-term remediation can be achieved with free laccase, it might consider-

ably reduce the cost. If the remediation is long-term, immobilized laccase should be used due to their better stability. Secondly, it is important to select the most suitable method of immobilization of laccase to reduce the loss of laccase as much as possible. The proper immobilization method with a good performance of the enzyme carrier could improve the efficiency of the enzyme. Finally, the existing enzyme carrier was processed and modified to ensure that it has a superior immobilization performance, which would be a trend in the development of enzyme carriers. As the application of immobilized laccase continues to expand, more new laccase carriers that meet the technological requirements and economic changes will be developed in the years to come.

## ИЗВОД

РАЗЛАГАЊЕ ПОЛИЦИКЛИЧНИХ АРОМАТИЧНИХ УГЉОВОДОНИКА У  
КОНТАМИНИРАНОМ ЗЕМЉИШТУ ИМОБИЛИЗОВАНОМ ЛАКАЗОМ

XIN WANG, SHI YU SUN, ZI JUN NI, ZHAO XING LI и JIA BAO

*School of Science, Shenyang University of Technology, Shenyang, China*

У раду је описан поступак биоразлагања полицикличних ароматичних угљоводоника (РАН) у земљишту применом лаказе. Мала активност слободне лаказе ограничава њену примену у биоремедијацији животне средине. У овом раду је коришћена лаказа из гљива, имобилизована методом адсорпције/умрежавања на најлонској мрежи или на хитозану, за разградњу РАН једињења у загађеном земљишту. Имобилизована лаказа је коришћена за разлагање пирена (Pyr) и бензо[а]пирена (BaP), а ефикасност разлагања је праћена на различитим температурама и рН. Резултати су показали да имобилизована лаказа разлаже Pyr и BaP 10–30 % ефикасније од слободне лаказе. Такође, ефикасност разлагања је већа када је лаказа имобилизована на хитозану него на најлонском носачу. Имобилизована лаказа је најефикаснија на 40 °С и на рН 4.

(Примљено 4. октобра 2017, ревидирано 15. јануара, прихваћено 22. јануара 2018)

## REFERENCES

1. T. Kudanga, G. S. Nyanhongo, G. M. Guebitz, S. Burton, *Enzyme Microb. Technol.* **48** (2011) 195
2. K. H. Kim, S. A. Jahan, E. Kabir, R. J. Brown, *Environ. Int.* **60** (2013) 71
3. C. T. Pham, T. Kameda, A. Toriba, K. Hayakawa, *Environ. Pollut.* **183** (2013) 175
4. D. Zhang, T. An, M. Qiao, B. G. Loganathan, X. Zeng, G. Sheng, J. Fu, *J. Hazard. Mater.* **192** (2011) 1
5. J. Feng, X. Li, J. Zhao, J. Sun, *Environ. Sci. Pollut. Res.* **24** (2017) 18195
6. G. Eibes, C. McCann, A. Pedezert, M. T. Moreira, G. Feijoo, J. M. Lema, *Biochem. Eng. J.* **51** (2010) 79
7. K. H. Jung, B. Yan, S. N. Chillrud, F. P. Perera, R. Whyatt, D. Camann, P. L. Kinney, R. L. Miller, *Int. J. Environ. Res. Public Health* **7** (2010) 189
8. C. P. Kaushik, A. K. Haritash, *Our Earth* **3** (2006) 1
9. H. I. Abdel-Shafy, M. Mansour, *Egypt. J. Pet.* **25** (2016) 107
10. G. Y. Gerasimov, *High Energy Chem.* **44** (2010) 1
11. N. N. Pozdnyakova, *Biotechnol. Res. Int.* **6** (2012) 1
12. C. García-Delgado, N. Jiménez-Ayuso, I. Frutos, A. Gárate, E. Eymar, *Environ. Sci. Pollut. R.* **20** (2013) 8690

13. M. Keshavarzifard, M. P. Zakaria, *Environ. Forensics* **16** (2015) 322
14. H. Yoshida, *J. Chem. Soc.* **43** (1883) 427
15. M. Keshavarzifard, M. P. Zakaria, T. S. Hwaib, F. M. Yusuff, S. Mustafa, *Environ. Sci. Pollut. Res.* **22** (2015) 9424
16. R. Hunsu-Udom, L. Jarupan, N. Poovarodom, T. Jinkarn, *Food. Agric. Org. (FAO)* **29** (2009) 146
17. M. T. Cambria, Z. Minniti, V. Librando, A. Cambria, *Appl. Biochem. Biotechnol.* **149** (2008) 1
18. Y. Wu, Y. Teng, Z. Li, X. Liao, Y. Luo, *Soil Biol. Biochem.* **40** (2008) 789
19. Y. Wang, X. Chen, J. Liu, F. He, R. Wang, *Environ. Sci. Pollut. R.* **20** (2013) 6222
20. A. S. Tsibart, A. N. Gennadiev, *Eurasian Soil Sci.* **46** (2013) 728
21. L. F. Bautista, G. Morales, R. Sanz, *Bioresour. Technol.* **101** (2010), 8541
22. D. N. Gabov, V. A. Beznosikov, *Eurasian Soil Sci.* **47** (2014) 18
23. G. Songulashvili, G. A. Jimenéz-Tobón, C. Jaspers, M. J. Penninecx, *Fungal Biol.* **116** (2012) 883
24. M. Fernández-Fernández, M. Á. Sanromán, D. Moldes, *Biotechnol. Adv.* **31** (2013) 1808
25. S. Georgieva, T. Godjevargova, D. G. Mita, N. Diano, C. Menale, C. Nicolucci, C. Romano Carratelli, L. Mita, E. Golovinsky, *J. Mol. Catal., B: Enzym.* **66** (2010) 210
26. A. Kandelbauer, O. Maute, R. W. Kessler, A. Erlacher, G. M. Gübitz, *Biotechnol. Bioeng.* **87** (2004) 552
27. M. Y. Arica, B. Altintas, G. Bayramoglu, *Bioresour. Technol.* **100** (2009) 665
28. S. Georgieva, T. Godjevargova, M. Portaccio, M. Lepore, D. G. Mita, *J. Mol. Catal., B: Enzym.* **55** (2008) 177
29. C. Prasse, W. Zech, F. Itanna, B. Glaser, *Toxicol. Environ. Chem.* **94** (2012) 1954
30. M. Mureşeanu, V. Pârvulescu, R. Ene, N. Cioateră, T. D. Pasatoiu, M. Andruh, *J. Mater. Sci.* **44** (2009) 67
31. B. Mahanty, K. Pakshirajan, V. V. Dasu, *Appl. Biochem. Biotechnol.* **134** (2006) 129
32. A. K. Haritash, C. P. Kaushik, *J. Hazard. Mater.* **169** (2009) 1
33. K. Hussain, R. R. Hoque, *Chemosphere* **119** (2015) 794
34. C. Thion, A. Cebron, T. Beguiristain, C. Leyval, *Biodegradation* **24** (2013) 569
35. G. Zafra, Á. E. Absalón, M. C. Cuevas, D. V. Cortés-Espinosa, *Water, Air, Soil Pollut.* **225** (2014) 1826.